SAN JUAN CREEK WATERSHED BACTERIAL STUDY

Final Report

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San Diego Region
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EXECUTIVE SUMMARY

In 1998, the lower reach of San Juan Creek (SJC) was listed by the Regional Water Quality Control Board (RWQCB) as water quality impaired in accordance with Section 303(d) of the Clean Water Act due to high levels of fecal indicator bacteria. Thus, in May 2000, the State Water Resources Control Board (through the San Diego RWQCB) provided funding to the County of Orange Public Facilities and Resources Department (PFRD) to perform a study in collaboration with the Orange County Health Care Agency (OCHCA) of the existing bacterial contamination within the San Juan Creek watershed.

San Juan Creek empties into Doheny Beach, which is also frequently posted as exceeding State recreational water quality standards. Orange County Public Health Laboratory (OCPHL) of the OCHCA was subcontracted by PFRD to carry out a bacterial watershed study of SJC to provide the County of Orange with information on the relative magnitudes of the bacterial loadings and sources to develop and implement an effective correction program using a combination of bacteriological monitoring surveys and bacterial source tracking (BST) analysis. The objectives of the sampling study were as follows:

1. Perform a bacterial survey of the water quality of the San Juan Creek watershed under dry weather conditions and locate any areas frequently exceeding bacteriologic water quality standards. Conduct a detailed monitoring survey of the problem areas identified.

2. Determine the source of the indicator bacteria found in the problem areas using bacterial source tracking techniques.

3. Compare two different techniques of bacterial source tracking, ribotyping and Antibiotic Resistance Analysis (ARA) to determine the accuracy of these techniques.

OCPHL collected water samples at various locations throughout the watershed to determine bacterial densities in creeks, storm drains and ocean water and for source identification testing. *E. coli* and *Enterococcus* were isolated from fecal and water samples and sent to reference laboratories to conduct ARA and ribotyping. The data from the bacterial monitoring and BST testing would be used to determine the sources of fecal pollution in SJC including humans, sewage, dogs, cats, horses and seagulls. However, since BST techniques are still in the developmental stages, OCPHL conducted a quality assurance (QA) study with the reference laboratories to determine the accuracy level of ARA and ribotyping prior to using these methods to test SJC watershed samples. This report describes the results of the SJC watershed bacterial monitoring and

source tracking study that was conducted in three phases to accomplish the following tasks:

Phase I (Task 3): Bacteriological Survey of Watershed and Adjacent Beach Recreational Water.

Thirty-six sampling locations, including 26 creeks, 7 storm drains, and 3 ocean sites, were sampled weekly for 11 weeks to identify areas which frequently exceed bacteriologic water quality standards. Water samples were tested for fecal indicator bacteria including total coliforms, fecal coliforms and *Enterococcus*.

Moderate to high levels of fecal indicator bacteria were detected in storm drains and creeks. The highest concentrations of fecal coliforms and *Enterococcus* were found in the storm drains as compared to the creeks and ocean sampling sites. Samples taken from creek sites distant to human habitat also had low to moderate levels of bacteria, suggestive of fecal contamination by non-human sources. The results of Phase I are described further in Chapter 1, "Phase I: Bacteriological Survey of San Juan Creek Watershed".

Phase II (Task 4): Detailed Bacteriological Survey of Identified Problem Areas.

Five sites were selected under the criteria previously described for continued monitoring and source tracking studies:

Pacific Ocean at the mouth of SJC (station number SJ02);
East side of SJC, at the beach, behind the berm (SJC2);
SJC below Pacific Coast Highway (PCH) (SJ06);
SJC above Trabuco Creek (SJ10); and
Trabuco Creek (SJ25).

Fecal indicator levels were determined for 69 samples collected over a 13-week period. *E. coli* testing was added during Phase II since it is more specific than fecal coliforms as in indicator of fecal contamination and to obtain isolates for bacterial source tracking testing conducted during Phase III.

As in Phase I, the bacterial concentrations for fecal indicators were higher overall in San Juan and Trabuco creeks compared to levels detected in the ocean water samples. The lower SJC area below PCH was consistently polluted with higher concentrations of fecal coliforms, including *E. coli* and *Enterococcus*. Overall, the bacterial concentrations found during Phase II were higher than levels for Phase I (excluding the effects of rain). The task 4 results are presented in detail in Chapter 2, "Phase II: Detailed Bacteriological Survey of San Juan Creek Watershed".

Phase III (Task 5): Source Identification by ARA and Ribotyping: Library Preparation and Technique Accuracy Determination.

Recent studies have reported the use of source tracking methods such as ARA and ribotyping to determine sources of bacteriological contamination as being human or animal derived based on differences in antibiotic resistance patterns or ribotype profiles of fecal indicators.

In this study, *E. coli* and *Enterococcus* bacteria were isolated from known species, including humans and animals that may be major contributors to high fecal indicator levels in the watershed. The bacterial strains were used to construct large ARA and ribotyping databases or libraries representative of *E. coli* and *Enterococcus* strains from humans and animals in the SJC watershed area.

To date, source tracking methods have not been widely tested in the field or subjected to rigorous QA testing. Therefore, OCPHL conducted a QA study to assess the suitability of using ARA and ribotyping for source identification of watershed isolates to be conducted in Phase IV. Accuracy and reproducibility of both methods was evaluated using 100 organisms from known sources provided to the contract laboratories as "blind" or proficiency samples. Based on the proficiency testing of known *E. coli* isolates, only 29% and 27% were accurately classified into the source groups using ARA and ribotyping, respectively. As for *Enterococcus*, 46% of 99 isolates were accurately classified. The results and discussion of the data are described in Chapter 3, "Phase III: Final Source Identification Report".

Phase IV (Task 6): Source Identification by ARA and Ribotyping: Source Identification of Watershed Isolates.

The objectives of Phase IV were to conduct ARA and ribotyping analyses to determine the relative contributions of human, sewage, horse, cat, dog and seagull feces to the levels of *E. coli* and *Enterococcus* in the watershed. Since the accuracy testing results obtained during Phase III indicated that ARA and ribotyping currently lack the accuracy and reproducibility level required to determine the sources of bacterial pollution, Phase IV was not undertaken.

Conclusions

Bacterial Survey

- Bacterial pollution measured by standard fecal indicator organisms was ubiquitous in storm drains and creeks sampled in the San Juan Creek watershed. Overall water quality measured against REC-1 standards was poor. The levels of indicators varied by the type of sampling location. The highest levels were found in storm drains, followed by creek sites, with the lowest levels detected at ocean sites. It is not known if lower levels in creeks were due to dilution, predation by other organisms, attachment to surfaces or inactivation.

- Concentrations of indicator organisms at storm drains varied temporally and spatially, with levels at some drains up to one log higher throughout the sampling period.

- While this study did not involve sampling at all storm drains in the watershed, the data indicate that storm drains are the major source of dry weather pollution at sampling stations upstream of PCH and below the furthest sampling site.

- Concentrations of fecal coliforms in storm drains ranged from a geometric mean of 1,401 colony forming units (CFU)/100 ml for station SJ07 (Storm drain L01S09 at La Novia and San Juan Creek) to 15,919 CFU/ml for SJ11 (Storm drain L02P02) at Trabuco Creek. These levels are similar to those found in urban runoff in the Newport Beach and Aliso watersheds (unpublished reports). Mean concentrations of indicators detected during the sampling period are not indicative of large or moderate levels of direct sewage contamination (1 to 2 log higher than typical levels). However, occasional spikes in indicator levels were detected in some creek sites and storm drains. The data does not rule out dilution of sewage from leaking pipes, cross-connected lines or the occurrence of intermittent sewage spills.

- Fecal coliform and *Enterococcus* concentrations were markedly higher at the San Juan Creek sampling sites near PCH as compared to sites further upstream. Possible explanations for this finding include indicator bacteria contribution from the intervening storm drain, direct contamination from waterfowl or other unidentified sources, and differences in stream morphology and ecology that allow organisms to regenerate.

- Low to moderate levels of bacteria were also found in creek sampling stations located distant to dense urbanization but within rural land use areas. This indicates that contamination is not limited to urban areas and that human land use activities as well as wildlife may be contributing sources at these sites.

- The concentrations of *Enterococcus* and fecal coliforms, two more specific indicators of fecal pollution than total coliforms, generally correlated by site. Levels of total coliforms often did not correlate with the other indictors.

- Rainfall events resulted in considerably higher levels of indicator bacteria at all the sites.

Bacterial Source Tracking

- In this study, the ARA and ribotyping methods did not demonstrate sufficient accuracy, discriminatory power, or reproducibility required to differentiate *E. coli* and *Enterococcus* isolates originating from human and non-human sources such as dogs, cats, horses and seagulls.

- Source tracking methods are emerging technologies that have not been rigorously tested. While they remain an area of research interest, they may have little or no use in determining the source of pollution in watersheds subject to multiple sources of contamination. Additional investigation is needed to address critical factors such as the monitoring design, type of indicator bacteria used, size and representativeness of the database, number of fecal indicator sources, number of proficiency test samples, type of data analysis used to interpret source identification results, bacterial variation, and geographic differences.

- Until an accurate source tracking technique is found, determining sources of pollution should rely on detailed watershed and sub-watershed surveys using conventional techniques that have been well established.

- Further studies are needed to validate source tracking methods using quality assurance testing.

CHAPTER 1

PHASE I: BACTERIOLOGICAL SURVEY

OF

SAN JUAN CREEK WATERSHED

Contents

1. Introduction

The San Juan Creek (SJC) Watershed comprises 103,683 acres in southern Orange County, California. The watershed contains areas with varying levels of development including undeveloped, suburban, and urban areas. SJC empties into the Pacific Ocean at Doheny Beach State Park in the city of Dana Point. The ocean water of this beach often fails California recreational water standards and is one of the beaches most often posted by the County Environmental Health Department as failing State recreational water standards. The outflow from SJC is assumed to be the source of the bacteria causing the failures of these standards.

To characterize the bacteriology of the SJC Watershed and to determine the sources of bacterial pollution, the San Diego Region of the California Regional Water Quality Board is funding a bacteriological study that includes bacteriologic surveys and bacteriological source tracking studies. This report includes the results of the first phase of the study, a bacteriologic survey of the three major creeks in the watershed, storm drains draining into the creeks and ocean water samples at the mouth of SJC.

2. Experimental Design / Sampling Plan

Sampling sites were selected to obtain a representative sample of ocean, creek and storm drains in the watershed. The total watershed was divided into 4 sub-watersheds. SJC watershed starts at the ocean and includes the ocean samples at the mouth of the creek. Trabuco Creek watershed starts at the confluence with SJC and Oso Creek watershed starts at the confluence with Trabuco Creek. Wagon Wheel canyon is a sub-watershed of SJC but is far upstream. Sampling sites were selected in SJC, Trabuco Creek, Oso Creek, the three major creeks in the watershed and Wagon Wheel Creek and storm drains flowing into these creeks. A total of 36 sites were chosen to represent the diversity of the watershed and types of storm drain systems and to accurately determine concentrations of indicator bacteria in the creeks. This resulted in 26 creek sites, 7 storm-drain sites and 3 ocean sites. There were 16 sites in the San Juan watershed, 10 sites in the Trabuco watershed 10 sites in the Oso watershed and one site in the Wagon Wheel watershed. Table 1, “San Juan Watershed Sampling Sites” contains information about each site including station number, location, latitude and longitude and the distance upstream from the mouth of the creek. The maps indicate the location of each sampling point. Sampling was carried out once a week beginning on April 30 ending July 10, 2001 for a total of 11 sampling weeks.

Each sample was collected and tested for total coliforms, fecal coliforms, and enterococcus utilizing the membrane filtration test (MF) as specified in Standard Methods for Examination of Water and Wastewater, 20th edition. The Orange County Public Health Laboratory, which is approved by California Environmental Laboratory Accrediting Program (ELAP), performed all sample collection and testing. Lack of water flow, schedules of other organization's collection personnel and inhibition of the test resulted in missing data points shown as blanks in the data tables.

3. Results

Bacterial levels in Watershed

The bacterial concentrations (the log of the concentrations and the log mean of all samples) for each station by sub-watershed by sampling week are presented in Table 2 "San Juan Watershed Study Bacterial Concentrations." For most sites flow was sufficient to collect samples all 11 weeks. Three sites were flowing only for the first few weeks of the study. This includes the two highest sites in the Trabuco watershed (SJ 17 and SJ 29) and the Via Angelina Storm Drain at Oso Creek (SJ 19). One site (SJ 11) was intermittent, 7 of 11 weeks were collected.

A 5-point summary of bacterial concentrations by station by watershed mile is presented in Figures 1A-C for the San Juan watershed, Tables 2A-C for the Trabuco watershed and Tables 3A-C for the Oso Watershed. This summary includes the minimum value, 25th percentile, median, log mean, 75th percentile and maximum value. Outliers, defined as values greater than 1.5 times the range between the 25th and 75th percentile are also indicated.

A summary of bacterial levels by three strata; ocean, creek and storm drain is presented in Figure 4. The bacterial levels were highest in storm drains and creeks and much lower in the ocean.

Comparison of bacterial concentrations to basin plan water quality standards

The bacterial levels measured were compared to REC-1 (Contact recreation) and REC-2 (Non-contact recreation) standards defined in the Water Quality Objectives of the Basin Plan. The REC-1 standard states that "the fecal coliform concentration based on a minimum of not less that five samples for any 30-day period, shall not exceed a log mean of 200/100 ml nor shall more than 10 percent of total samples collected during a 30-day period exceed 400/100 ml." To apply this standard to the study results, which were taken over a period of only 11 weeks, the log mean was calculated for the weeks in which there were at least 4 previous results available. The results are presented in Figure 5. "Compliance

with REC-1 Standard". Of the sites that had sufficient results for this analysis, only 3 sites were 100% compliant with the standard, one ocean site and 2 creek sites. Five of 6 storm drains and 11 of 19 creek sites had zero% compliance

The REC-2 standard states that "the average fecal coliform concentrations for any 30-day period, shall not exceed 2000/100ml nor shall more than 10 percent of samples collected during any 30-day period exceed 4000/100 ml." To apply this standard to the study results, which were taken over a period of only 11 weeks, the average was calculated for the weeks in which there were at least 4 previous results available. The results are presented in Figure 6. "Compliance with REC-2 Standard. Of the sites that had sufficient results for this analysis, 2 of 2 ocean sites, 14 of 21 creek sites and 0 of 6 storm drain sites were 100% compliant with the standard. 3 of 6 storm drain sites and 6 of 21 storm drain sites had zero% compliance.

4. Conclusions

1. Bacterial pollution measured by standard indicator organisms was ubiquitous in storm drains and creeks. Overall, storm drains had the highest concentration of indicator organisms, creeks had lower concentrations and ocean sites were even lower.

2. Concentrations of indicator organisms in storm drains and creeks were similar to what is expected from urban runoff. Levels indicative of large or moderate amounts of direct sewage contamination were not seen.

3. All storm drains tested had moderate to high concentrations of indicator organisms ranging from a log mean fecal coliform of 1401 for SJ07 (Storm drain L01S09 at La Novia and SJC) to 15,919 for SJ11 (Storm drain L02P02 at Trabuco Creek).

4. All creek sites tested had moderate concentrations of indicator organisms ranging from a log mean fecal coliform of 92 for SJ06 (SJC upstream of Trabuco Creek) to 3770 for SJ 22 (Oso Creek at Olympad above Drains).

5. The concentrations of the two more specific indicators of fecal pollution, enterococcus and fecal coliform, generally correlated by site. Levels of total coliforms often did not correlate.

6. Fecal coliform and enterococcus concentrations were markedly higher at the SJC sampling sites at PCH and below (SJ01, SJ02, SJC2 sites at creek behind beach and SJC2, SJC at PCH) compared to sites further upstream. Two possible explanations could be contamination from

intervening storm drain outlets such as SJ03 (L01S02 at SJC) or direct contamination from waterfowl.

7. Overall water quality measured against REC-1 standards was poor. Storm drains and creeks met REC-1 standards only rarely. Two creek samples had 100% compliance (SJ26, SJC downstream of Trabuco Creek and SJ06, SJC upstream of Trabuco Creek). The remainder of sites had 0% to 42% compliance. One storm drain had 14% compliance; the remainder of storm drains were 0% compliant.

8. While large storm drains had the highest concentration of indicator bacteria even small storm drains with intermittent flows were moderately contaminated. SJ19, (Via Angelina Storm drain at Oso Creek) which drains a small area, had moderate concentration of indicator organisms. When runoff was present, the log mean of fecal coliforms was 329 and *Enterococcus* was 809.

9. While urban runoff in storm drains was shown to contain bacterial contamination, there may also be a contribution by wildlife. The sample sites furthest upstream and most removed from human habitation had indications of low to moderate levels of bacterial pollution. SJ17 and SJ29 (Trabuco Creek above and below S19) had fecal coliform concentrations between 240 and 880 and enterococcus concentrations between 20 and 460. These sites only had flow for the first 3 weeks of the study before becoming dry. The two sites furthest upstream in SJC, SJ09 and SJ30 (SJC at Ortega Highway) had log mean fecal coliform concentrations of 292 and 583 and log mean *Enterococcus* concentrations of 801 and 1236.

Figure 1A - San Juan Watershed Total Coliforms

5-Point Summaries* and Means by Station

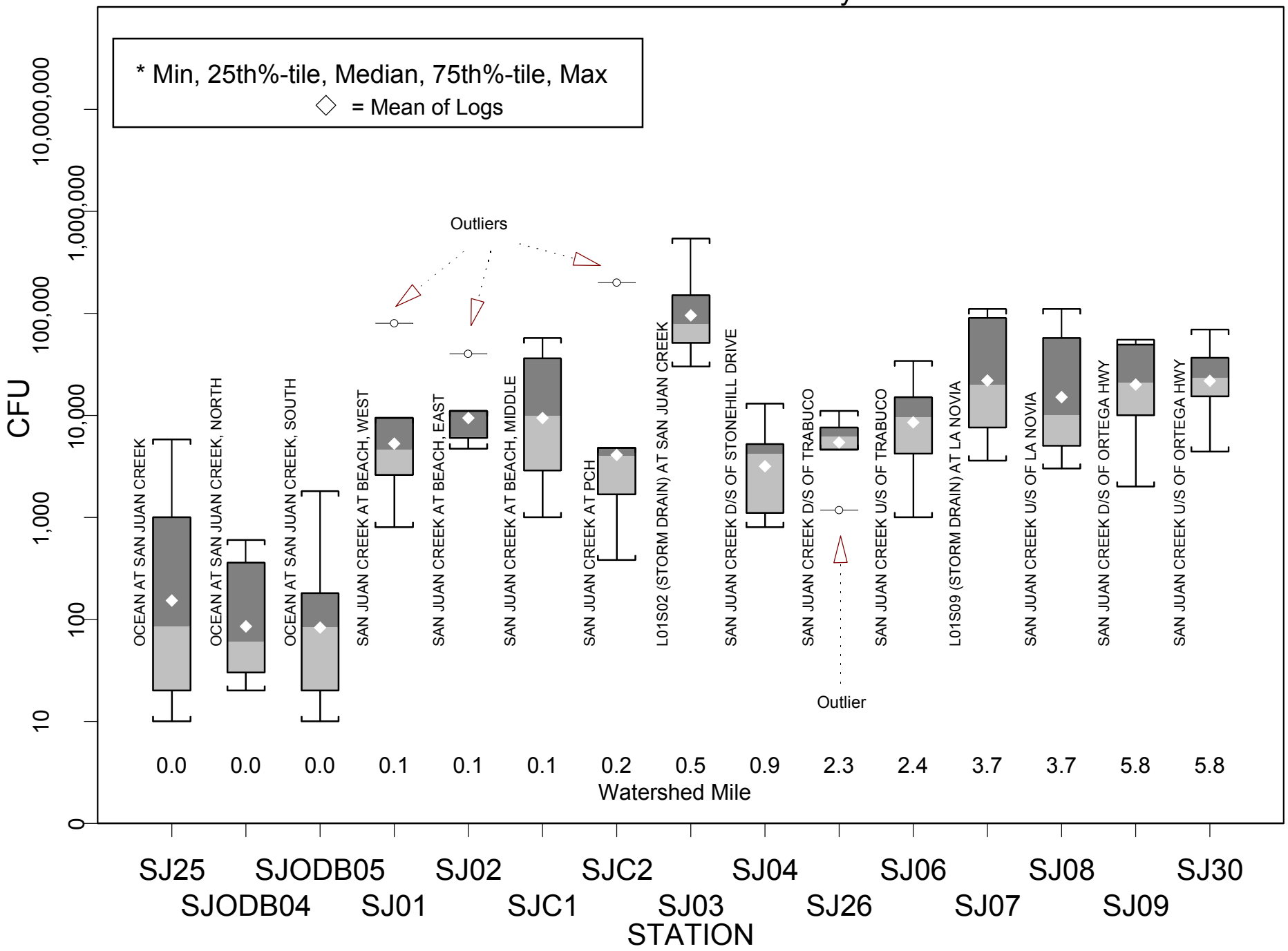


Figure 1B - San Juan Watershed Fecal Coliforms

5-Point Summaries* and Means by Station

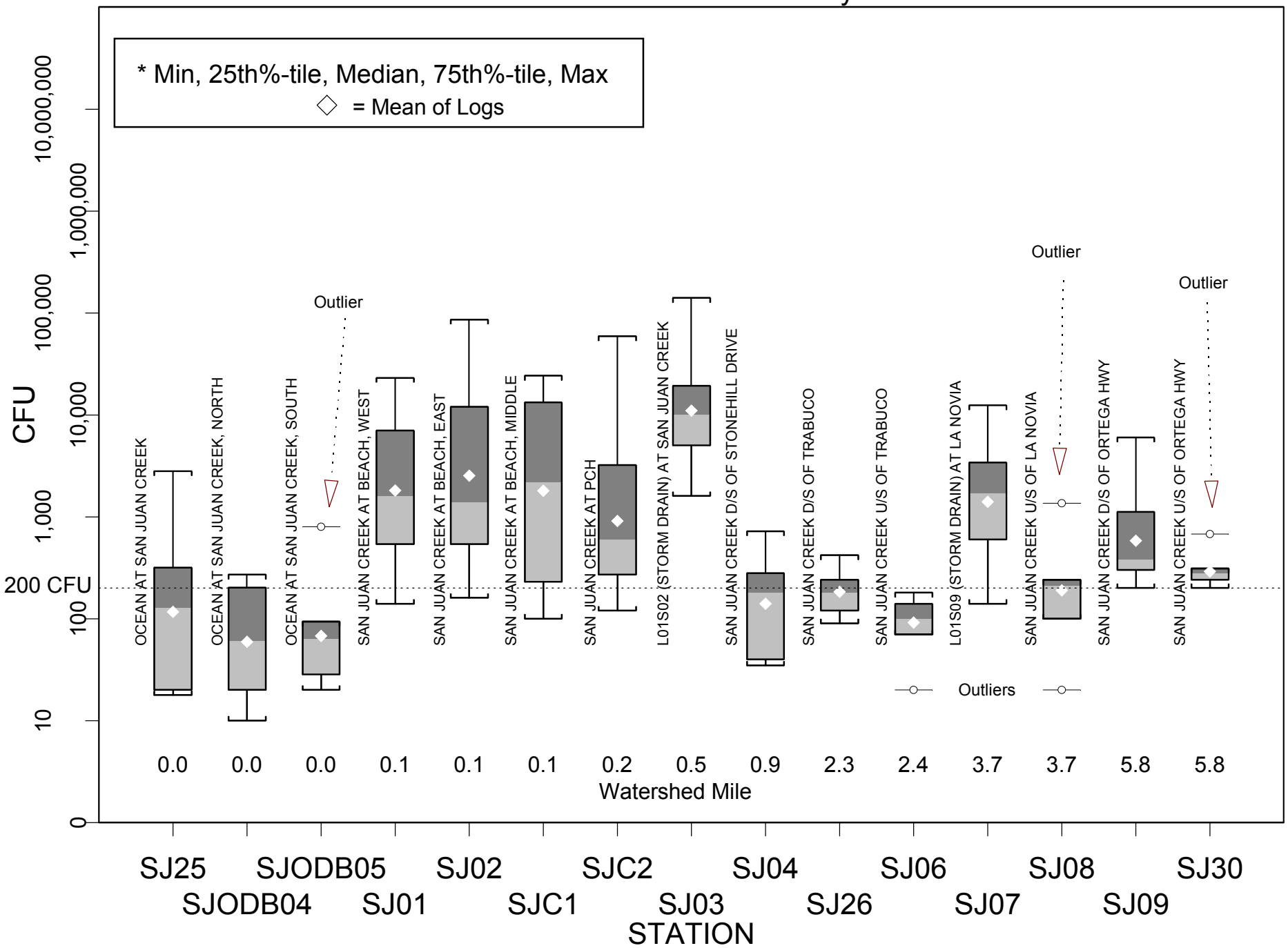


Figure 1C - San Juan Watershed Enterococcus

5-Point Summaries* and Means by Station

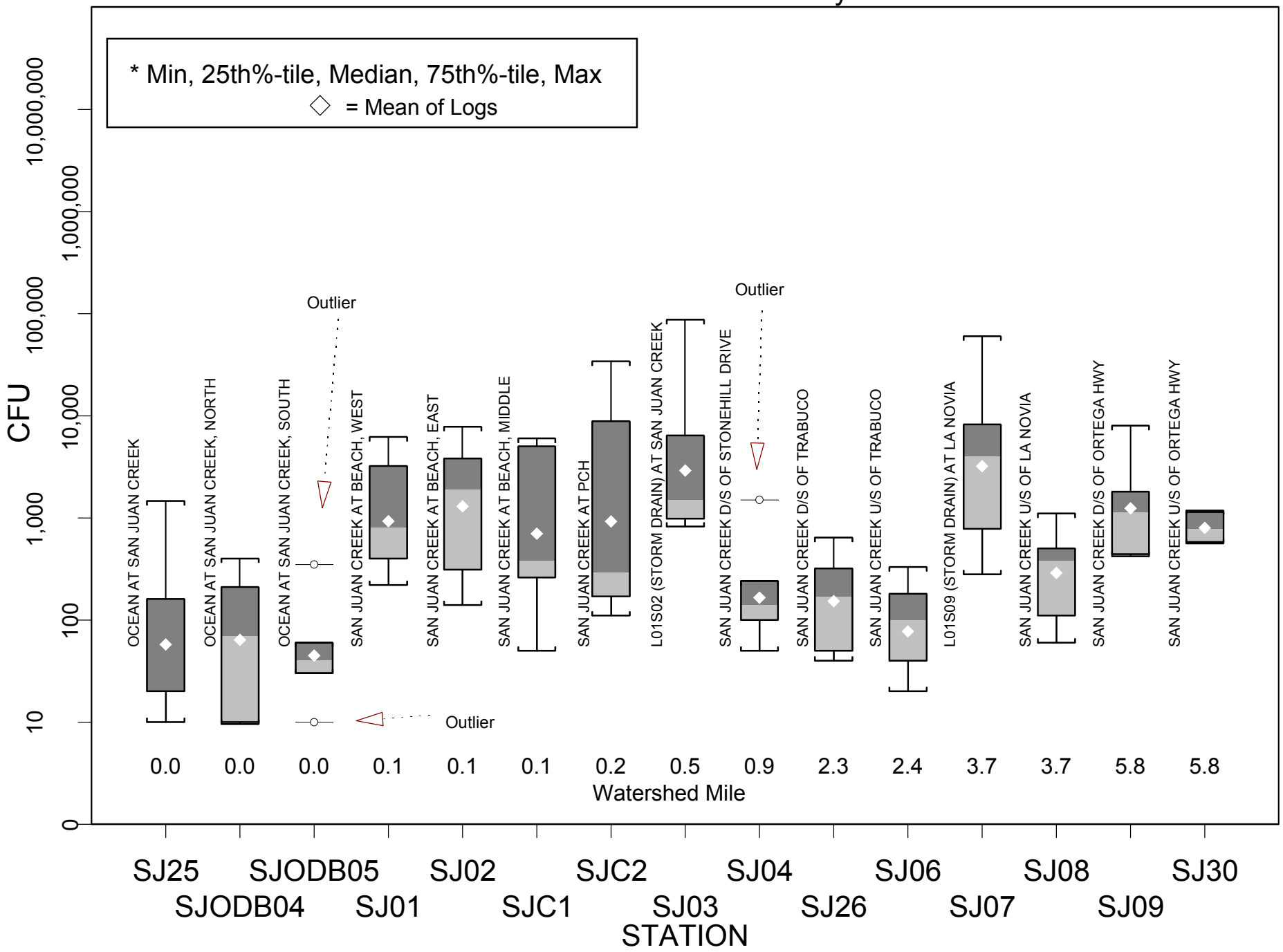


Figure 2A - Trabuco Watershed Total Coliforms

5-Point Summaries* and Means by Station

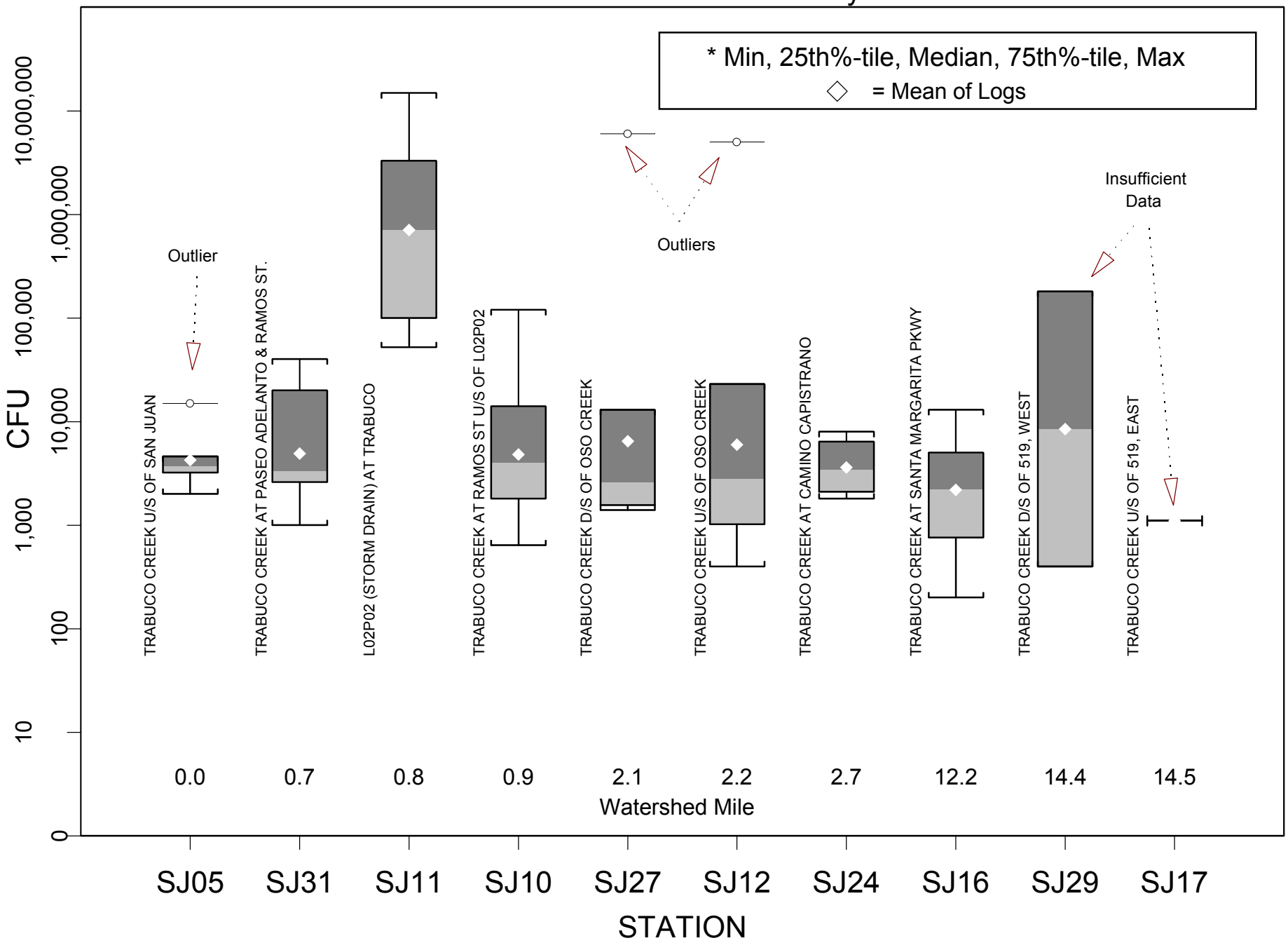


Figure 2B - Trabuco Watershed Fecal Coliforms

5-Point Summaries* and Means by Station

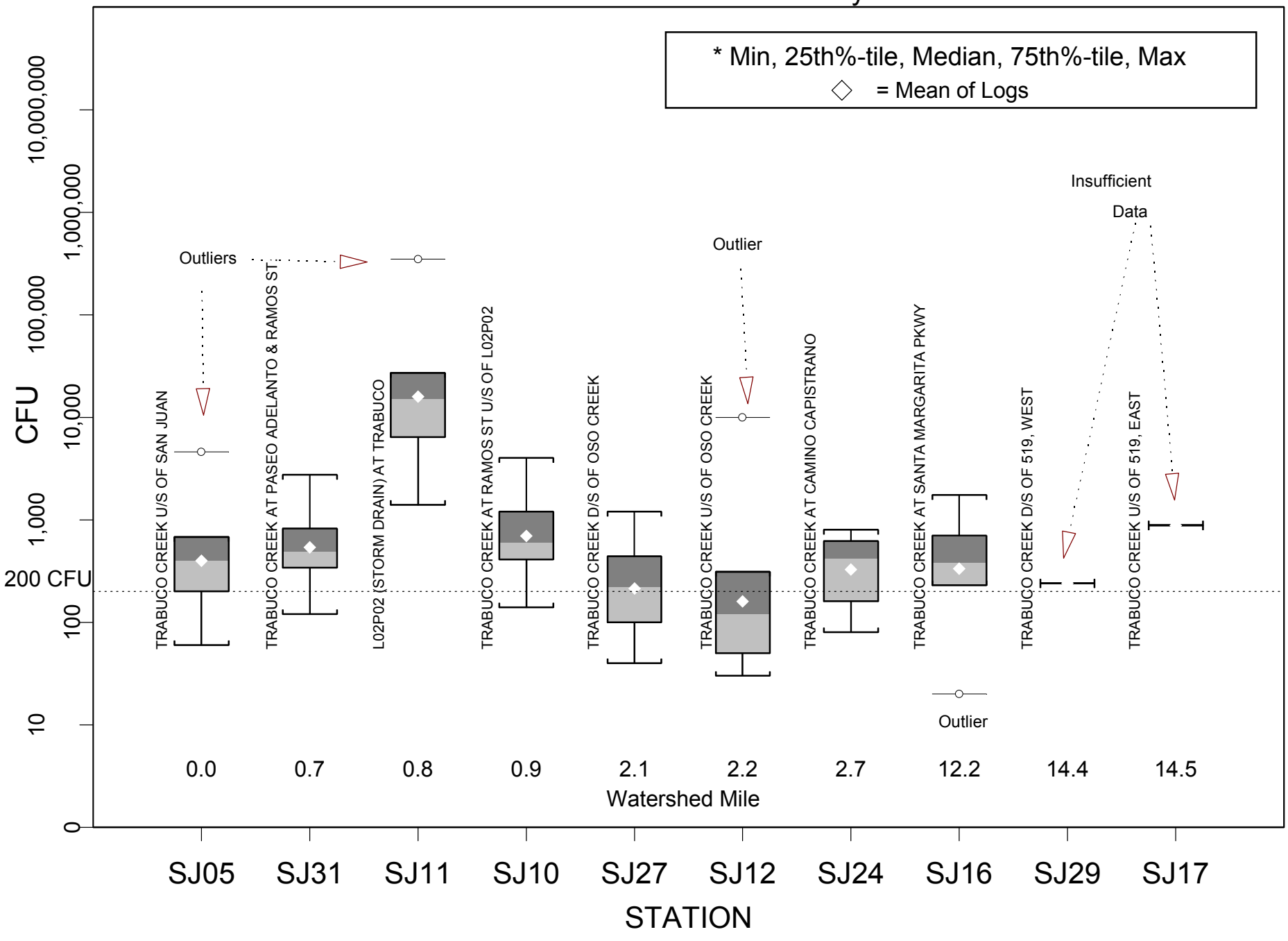


Figure 2C - Trabuco Watershed Enterococcus

5-Point Summaries* and Means by Station

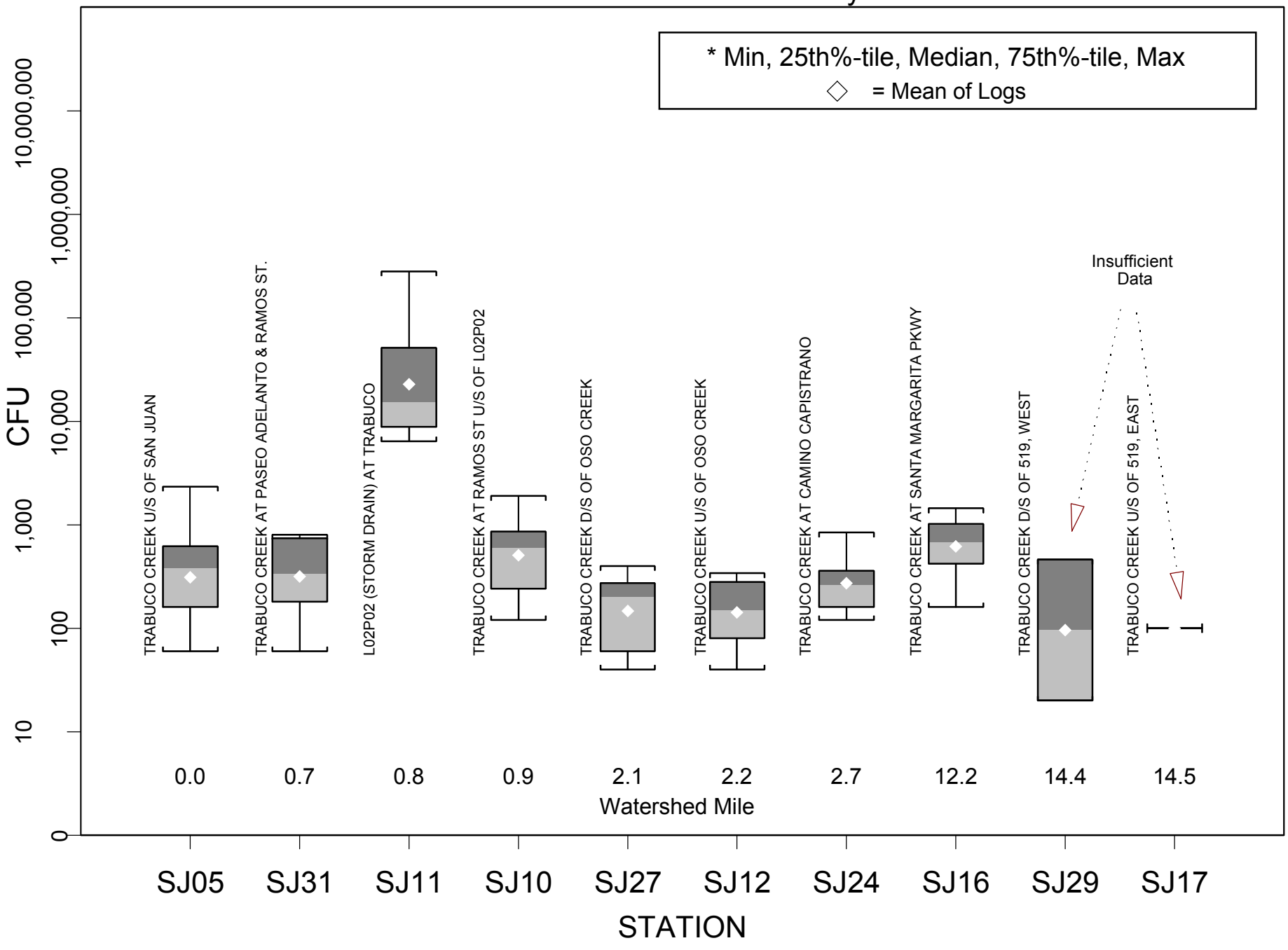


Figure 3A - Oso Watershed Total Coliforms

5-Point Summaries* and Means by Station

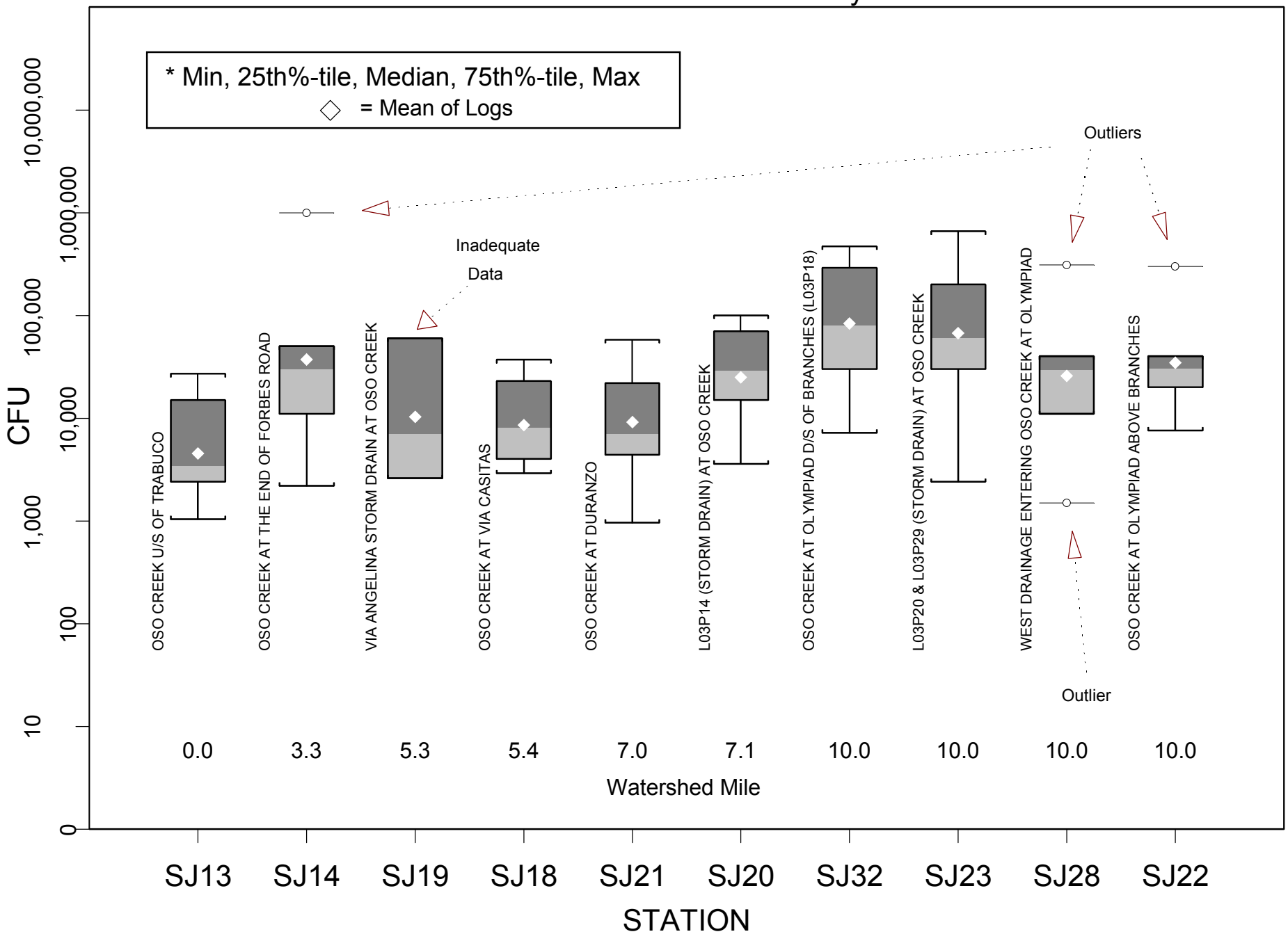


Figure 3B - Oso Watershed Fecal Coliforms

5-Point Summaries* and Means by Station

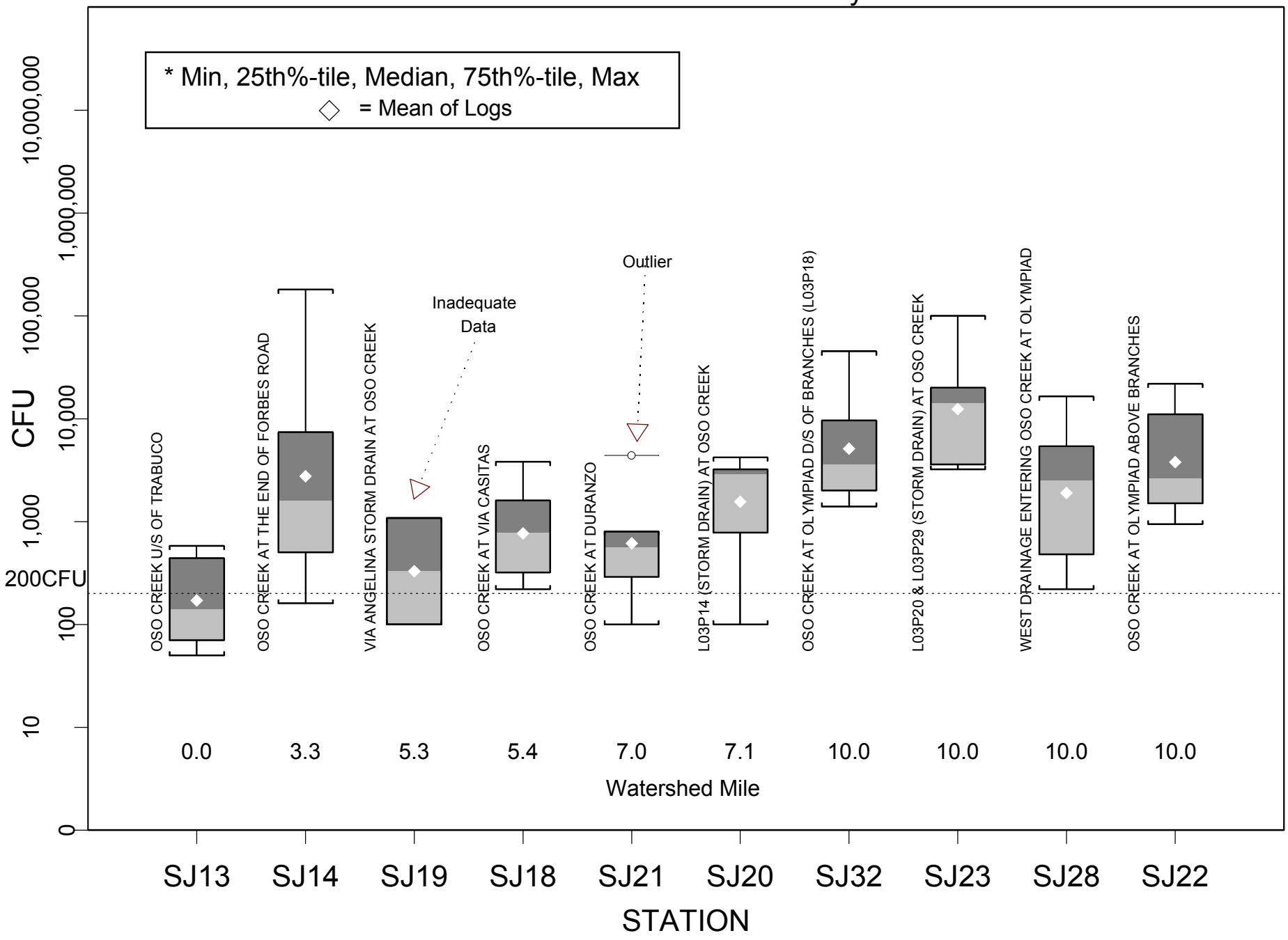


Figure 3C - Oso Watershed Enterococcus

5-Point Summaries* and Means by Station

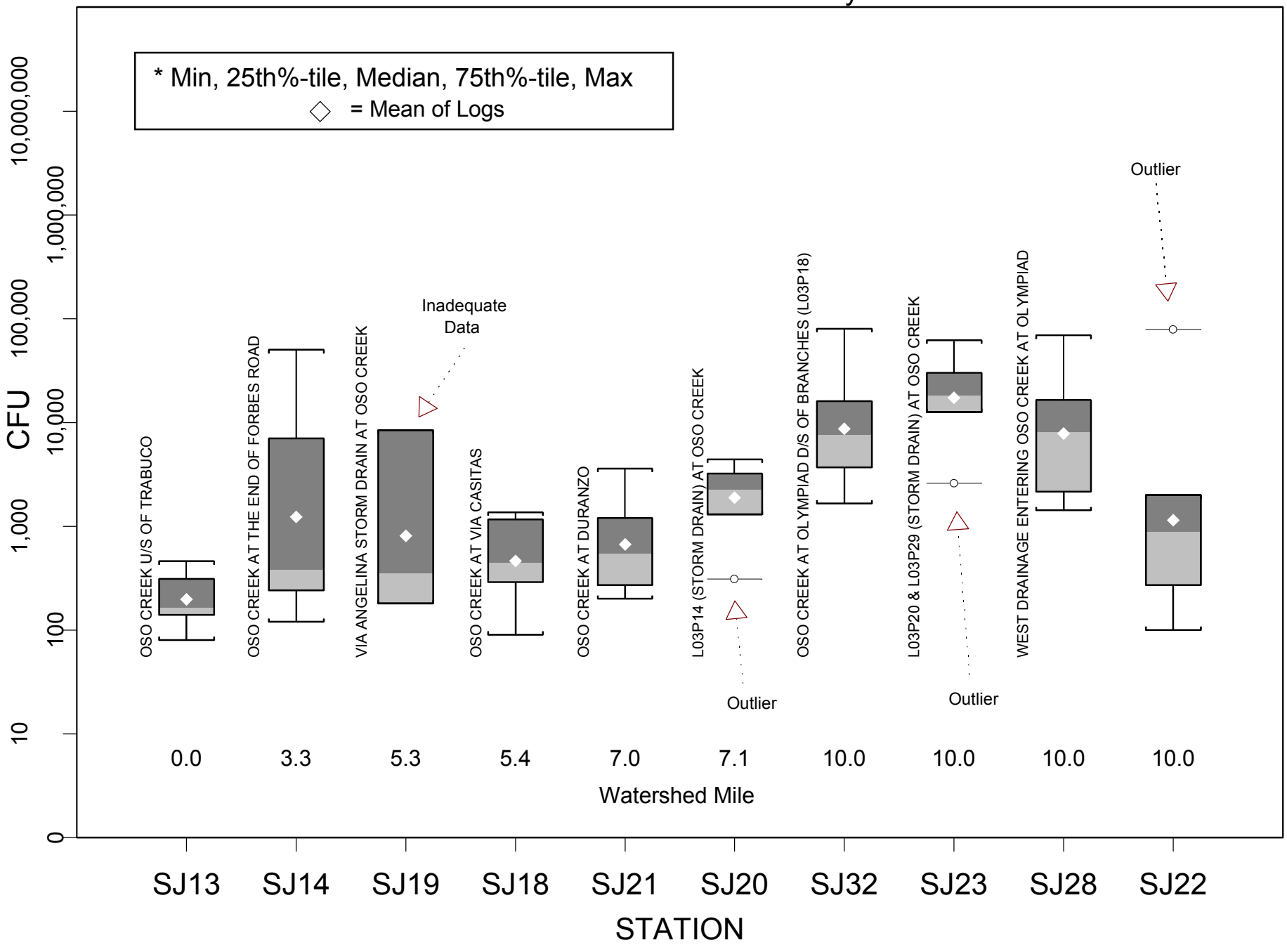


Figure 4 - TC, FC and Enterococcus

5-Point Summaries* and Means by Sample Type

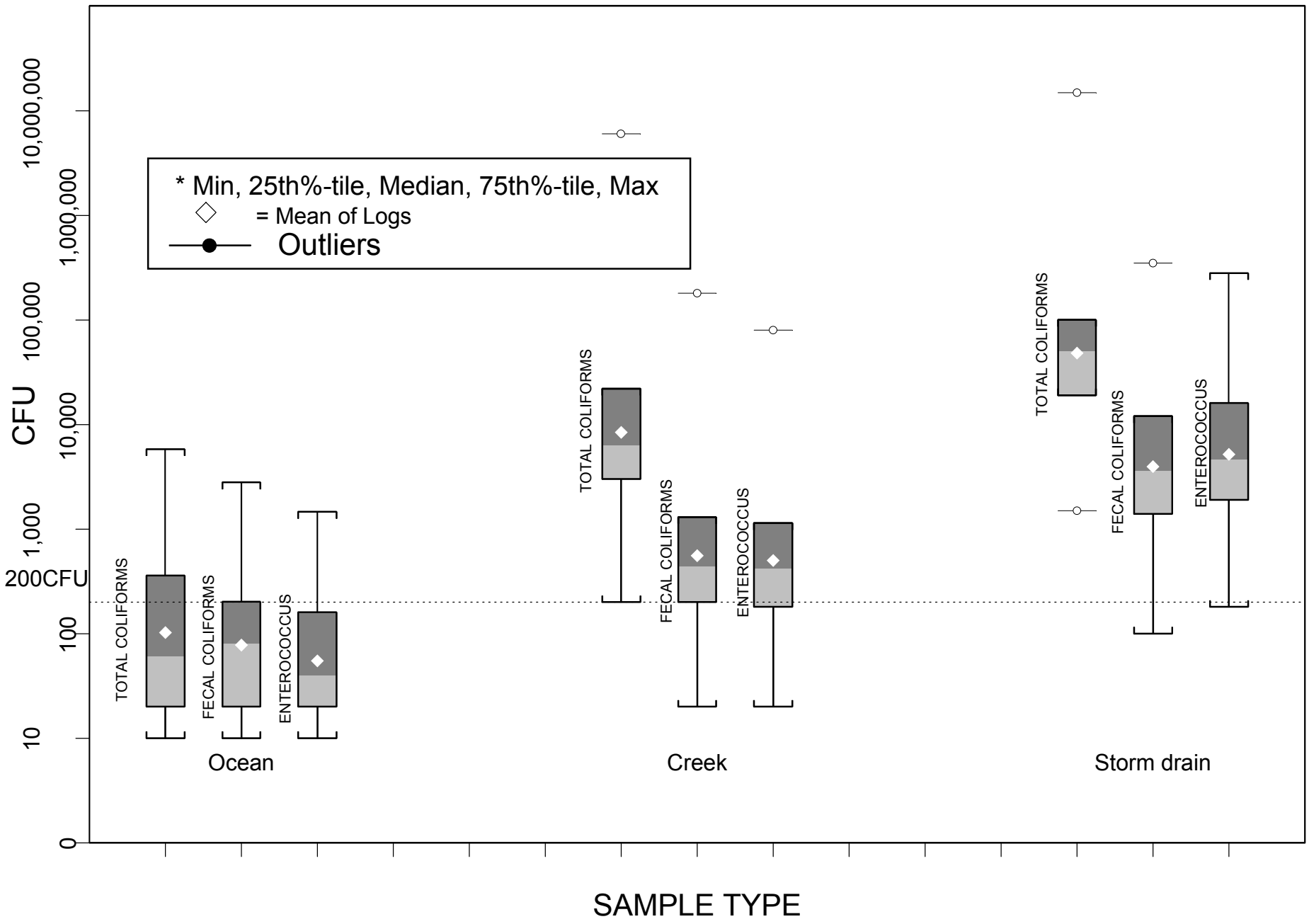


Figure 5 - Compliance With REC-1 Standard

Based on 5-Week Average < 200

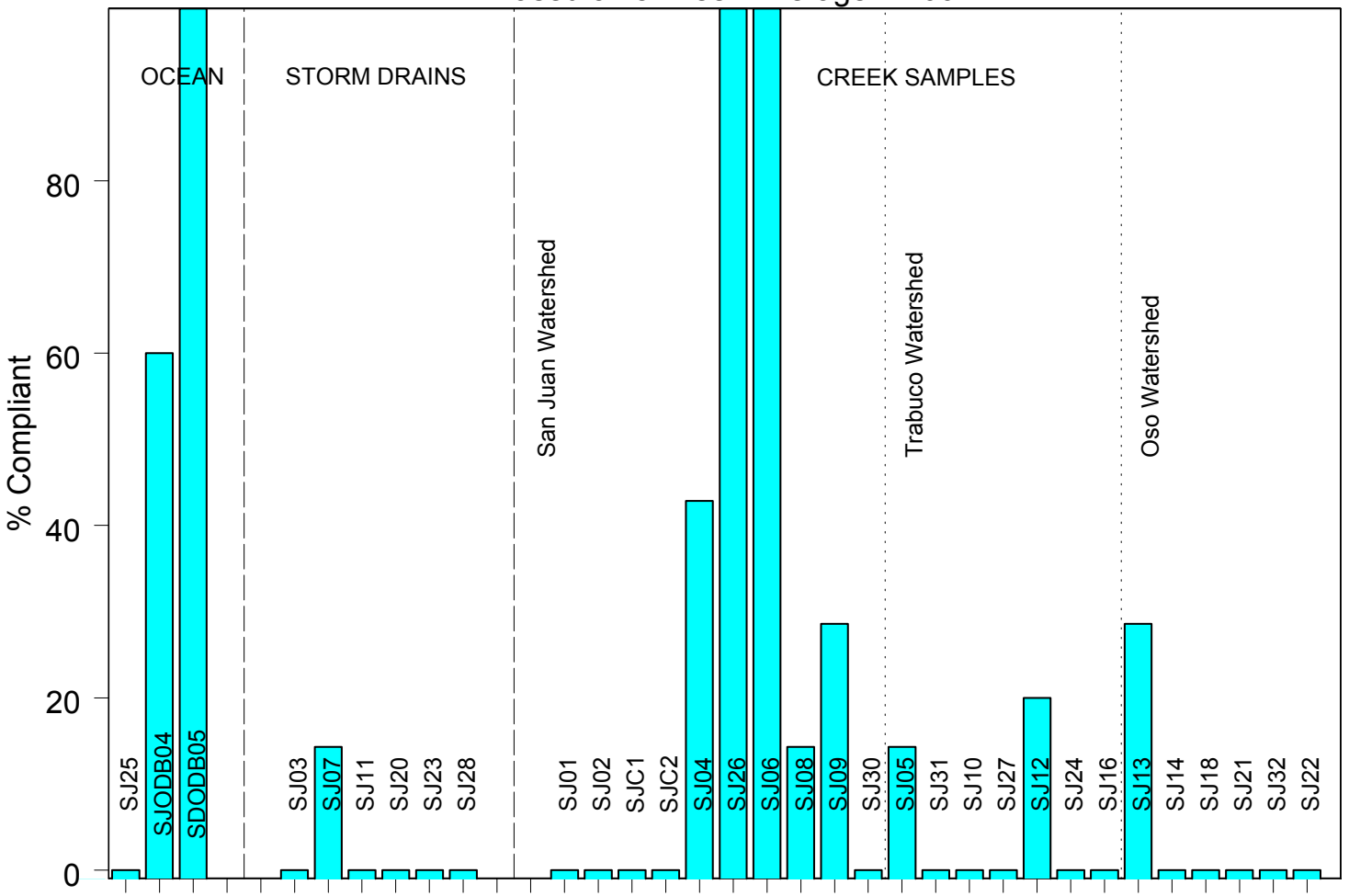


Figure 6 - Compliance With REC-2 Standard

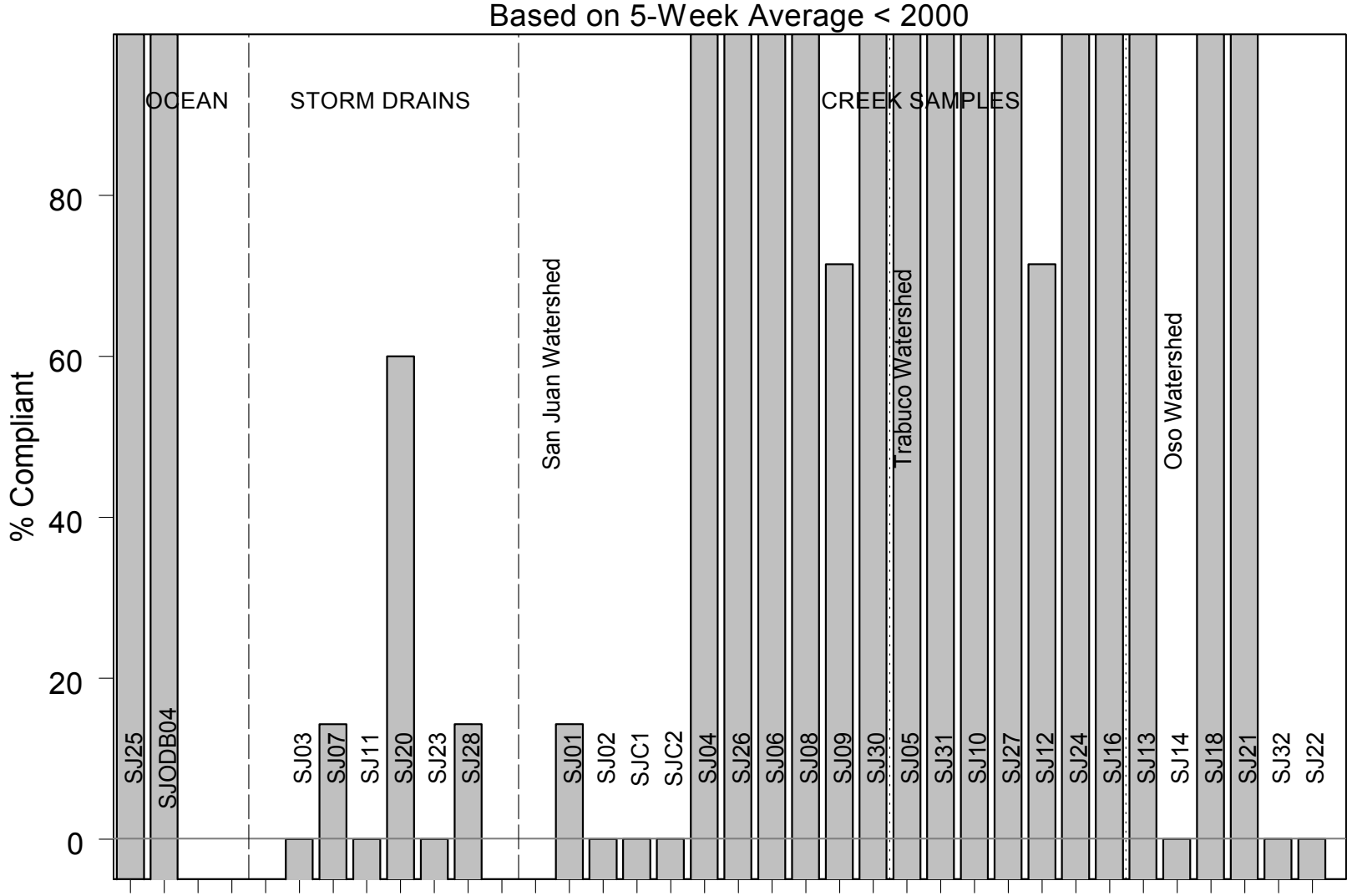


Table 1 - San Juan Watershed Study Sampling Sites

Watershed: *1) San Juan*

Watershed Mile	Station Number	Location	Type of Sample	Latitude/Longitude	Notes
0	SJ25	OCEAN AT SAN JUAN CREEK	Ocean	33 0 27.698 ' N / 117 0 40.933 ' W	Ocean at San Juan Creek Mouth
0	SJODB04	OCEAN AT SAN JUAN CREEK, NORTH	Ocean	0 ' / 0 '	Ocean 250' North of San Juan Creek Mouth
0	SJODB05	OCEAN AT SAN JUAN CREEK, SOUTH	Ocean	0 ' / 0 '	Ocean 250' South of San Juan Creek Mouth
0.05	SJ01	SAN JUAN CREEK AT BEACH, WEST	Creek	33 0 27.720 ' N / 117 0 41.044 ' W	Behind Berm, West
0.05	SJ02	SAN JUAN CREEK AT BEACH, EAST	Creek	33 0 27.722 ' N / 117 0 40.945 ' W	Behind Berm, East
0.05	SJC1	SAN JUAN CREEK AT BEACH, MIDDLE	Creek	33 0 27.720 ' N / 117 0 41.044 ' W	Behind Berm, Middle
0.2	SJC2	SAN JUAN CREEK AT PCH	Creek	33 0 27.812 ' N / 117 0 41.025 ' W	
0.5	SJ03	L01S02 AT SAN JUAN CREEK	Storm drain	33 0 27.927 ' N / 117 0 40.933 ' W	
0.9	SJ04	SAN JUAN CREEK D/S OF STONEHILL DRIVE	Creek	33 0 28.375 ' N / 117 0 40.788 ' W	
2.3	SJ26	SAN JUAN CREEK D/S OF TRABUCO	Creek	33 0 29.367 ' N / 117 0 39.946 ' W	
2.4	SJ06	SAN JUAN CREEK U/S OF TRABUCO	Creek	33 0 29.397 ' N / 117 0 39.950 ' W	
3.7	SJ07	L01S09 AT LA NOVIA	Storm drain	33 0 30.120 ' N / 117 0 38.887 ' W	
3.71	SJ08	SAN JUAN CREEK U/S OF LA NOVIA	Creek	33 0 30.142 ' N / 117 0 38.884 ' W	
5.8	SJ09	SAN JUAN D/S OF ORTEGA HWY	Creek	33 0 31.119 ' N / 117 0 37.500 ' W	
5.81	SJ30	SAN JUAN U/S OF ORTEGA HWY	Creek	33 0 31.136 ' N / 117 0 37.500 ' W	

Table 1 - San Juan Watershed Study Sampling Sites

Watershed: *2) Trabuco*

Watershed Mile	Station Number	Location	Type of Sample	Latitude/Longitude	Notes
0	SJ05	TRABUCO CREEK U/S OF SAN JUAN	Creek	33 0 29.416 ′N / 117 0 39.961 ′W	
0.7	SJ31	TRABUCO CREEK AT PASEO ADELANTO & RAMOS ST.	Creek	33 0 30.241 ′N / 117 0 40.052 ′W	
0.8	SJ11	L02P02 AT TRABUCO	Storm drain	33 0 30.241 ′N / 117 0 40.052 ′W	
0.9	SJ10	TRABUCO CREEK AT RAMOS ST. U/S OF L02P02	Creek	33 0 35.248 ′N / 117 0 39.741 ′W	
2.1	SJ27	TRABUCO CREEK D/S OF OSO CREEK	Creek	33 0 31.161 ′N / 117 0 40.399 ′W	
2.2	SJ12	TRABUCO CREEK U/S OF OSO CREEK	Creek	33 0 33.161 ′N / 117 0 40.399 ′W	
2.7	SJ24	TRABUCO CREEK AT CAMINO CAPISTRANO	Creek	33 0 31.533 ′N / 117 0 40.211 ′W	
12.2	SJ16	TRABUCO CREEK AT SANTA MARGARITA PARKWAY	Creek	33 0 38.276 ′N / 117 0 36.975 ′W	
14.4	SJ29	TRABUCO CREEK D/S OF S19, WEST	Creek	33 0 39.555 ′N / 117 0 35.174 ′W	
14.5	SJ17	TRABUCO CREEK U/S OF S19, EAST	Creek	33 0 39.566 ′N / 117 0 35.143 ′W	

Table 1 - San Juan Watershed Study Sampling Sites

Watershed: *3) Oso*

Watershed Mile	Station Number	Location	Type of Sample	Latitude/Longitude	Notes
0	SJ13	OSO CREEK U/S OF TRABUCO	Creek	33 0 33.161 ′ N / 117 0 40.400 ′ W	
3.3	SJ14	OSO CREEK AT THE END OF FORBES ROAD	Creek	33 0 33.710 ′ N / 117 0 40.572 ′ W	
5.3	SJ19	VIA ANGELINA STORM DRAIN AT OSO CREEK	Storm drain	33 0 35.233 ′ N / 117 0 39.767 ′ W	
5.4	SJ18	OSO CREEK AT VIA CASITAS	Creek	33 0 35.248 ′ N / 117 0 39.741 ′ W	
7	SJ21	OSO CREEK AT DURANZO	Creek	33 0 36.361 ′ N / 117 0 38.940 ′ W	
7.1	SJ20	L03P14 AT OSO CREEK	Storm drain	33 0 36.371 ′ N / 117 0 38.914 ′ W	
9.99	SJ32	OSO CREEK AT OLYMPAID D/S OF BRANCHES (L03P18)	Creek	33 0 38.297 ′ N / 117 0 38.335 ′ W	
10	SJ23	L03P20 & L03P29 AT OSO CREEK	Storm drain	33 0 38.300 ′ N / 117 0 38.342 ′ W	
10	SJ28	WEST DRAINAGE ENTERING OSO CREEK AT OLYMPIAD	Storm drain	33 0 38.301 ′ N / 117 0 38.345 ′ W	
10.01	SJ22	OSO CREEK AT OLYMPIAD ABOVE BRANCHES	Creek	33 0 38.306 ′ N / 117 0 38.345 ′ W	

Table 1 - San Juan Watershed Study Sampling Sites

Watershed: *4) Wagon Wheel*

Watershed Mile	Station Number	Location	Type of Sample	Latitude/Longitude	Notes
1.2	SJ15	WAGON WHEEL CREEK U/S OF CROSSING IN RILEY PARK	Creek	33 0 34.367 ′ N / 117 0 35.537 ′ W	Sub watershed of San Juan Creek

Table 2 - San Juan Watershed Study Bacterial Concentrations

Watershed: *1) San Juan*

Station Number SJ25 ***Location:*** OCEAN AT SAN JUAN CREEK ***Mile:*** 0

Indicator	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8	Week 9	Week 10	Week 11	Mean (Log)
LOG ENTEROCOCCUS	1.30	3.16	1.00	1.30	2.20	2.20	2.38	1.30	1.30	1.30	1.90	1.760
LOG FC		3.45	1.30	1.30	2.40	2.20	2.60	2.00		1.30		2.069
LOG TC	1.30	3.76	1.78		3.08	2.78	3.00	1.00	2.08	1.30	1.78	2.186
ENTEROCOCCUS	20	1460	10	20	160	160	240	20	20	20	80	58
FC		2800	20	20	250	160	400	100		20		117
TC	20	5800	60		1200	600	1000	10	120	20	60	153

Station Number SJODB04 ***Location:*** OCEAN AT SAN JUAN CREEK, NORTH ***Mile:*** 0

Indicator	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8	Week 9	Week 10	Week 11	Mean (Log)
LOG ENTEROCOCCUS	1.95	1.85	2.30	1.60	2.40	2.32	2.60	1.85	1.00	1.00	1.00	1.806
LOG FC	1.95	2.38			2.43	2.23	1.30	1.30	1.00		1.60	1.775
LOG TC	1.78	2.56	1.30	1.48	2.61	2.78	1.95	2.30	1.48	1.30	1.70	1.931
ENTEROCOCCUS	90	70	200	40	250	210	400	70	10	10	10	64
FC	90	240			270	170	20	20	10		40	60
TC	60	360	20	30	410	600	90	200	30	20	50	85

Table 2 - San Juan Watershed Study Bacterial Concentrations

Watershed: *1) San Juan*

Station Number SJODB05 ***Location:*** OCEAN AT SAN JUAN CREEK, SOUTH ***Mile:*** 0

Indicator	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8	Week 9	Week 10	Week 11	Mean (Log)
LOG ENTEROCOCCUS	1.60	2.00	1.60	1.48	1.60	1.30	2.54	1.78	1.48	1.00	1.78	1.651
LOG FC	2.04	1.90	1.60		1.70	1.30	2.90	1.30			1.90	1.832
LOG TC	2.45	2.20		1.30	2.26	2.15	3.26	1.60	1.30	1.00	1.70	1.921
ENTEROCOCCUS	40	100	40	30	40	20	350	60	30	10	60	45
FC	110	80	40		50	20	800	20			80	68
TC	280	160		20	180	140	1800	40	20	10	50	83

Station Number SJ01 ***Location:*** SAN JUAN CREEK AT BEACH, WEST ***Mile:*** 0.05

Indicator	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8	Week 9	Week 10	Week 11	Mean (Log)
LOG ENTEROCOCCUS	3.79	3.51	3.11	2.90	2.60	2.93	2.64	3.51	2.87	2.45	2.34	2.969
LOG FC	3.85	4.06	3.60	2.15	3.20	3.15	2.73	4.36	3.60	2.26	2.90	3.260
LOG TC	4.34		3.60	3.97	3.41	3.72	2.90	4.90	3.90	3.00	3.48	3.723
ENTEROCOCCUS	6200	3200	1300	800	400	860	440	3200	740	280	220	931
FC	7000	11400	4000	140	1600	1400	540	23000	4000	180	800	1818
TC	22000		4000	9400	2600	5200	800	80000	8000	1000	3000	5291

Table 2 - San Juan Watershed Study Bacterial Concentrations

Watershed: *1) San Juan*

Station Number SJ02 ***Location:*** SAN JUAN CREEK AT BEACH, EAST ***Mile:*** 0.05

Indicator	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8	Week 9	Week 10	Week 11	Mean (Log)
LOG ENTEROCOCCUS	3.83	3.58	3.89	2.49	2.70	3.26	3.30	3.45		2.48	2.15	3.112
LOG FC	3.88	4.08	4.93	2.20	2.85	2.78	3.45	4.49		2.64	2.73	3.404
LOG TC	4.04		4.36	3.79	3.67	3.70	4.04	4.60		3.78	3.78	3.974
ENTEROCOCCUS	6800	3800	7800	310	500	1800	2000	2800		300	140	1295
FC	7600	12000	86000	160	700	600	2800	31000		440	540	2533
TC	11000		23000	6200	4700	5000	11000	40000		6000	6000	9420

Station Number SJC1 ***Location:*** SAN JUAN CREEK AT BEACH, MIDDLE ***Mile:*** 0.05

Indicator	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8	Week 9	Week 10	Week 11	Mean (Log)
LOG ENTEROCOCCUS	3.76	3.78	3.53	2.43	2.08	2.85	2.48	3.70	1.70	2.58	2.41	2.845
LOG FC	4.12	4.38	3.87	2.00	2.41	3.34	4.26	4.05	2.08	2.36	2.94	3.257
LOG TC	4.37			3.66	3.99	4.00	4.76	4.75	3.26		3.00	3.972
ENTEROCOCCUS	5800	6000	3400	270	120	700	300	5000	50	380	260	700
FC	13200	24200	7400	100	260	2200	18400	11200	120	230	870	1807
TC	23200			4600	9800	10000	57000	56000	1800		1000	9383

Table 2 - San Juan Watershed Study Bacterial Concentrations

Watershed: *1) San Juan*

Station Number SJC2 ***Location:*** SAN JUAN CREEK AT PCH ***Mile:*** 0.2

Indicator	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8	Week 9	Week 10	Week 11	Mean (Log)
LOG ENTEROCOCCUS	2.20	3.76	3.94	4.11	2.46	2.34	2.23	4.53	2.23	2.04	2.76	2.966
LOG FC	2.77	3.51	4.20	2.90	2.20	2.43	2.08	4.77	2.43	2.78	2.48	2.959
LOG TC		3.66			2.58	3.00	3.70	5.30	3.60	3.60	3.45	3.612
ENTEROCOCCUS	160	5800	8800	13000	290	220	170	34000	170	110	580	925
FC	590	3200	15800	800	160	270	120	59000	270	600	300	910
TC		4600			380	1000	5000	200000	4000	4000	2800	4090

Station Number SJ03 ***Location:*** L01S02 AT SAN JUAN CREEK ***Mile:*** 0.5

Indicator	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8	Week 9	Week 10	Week 11	Mean (Log)
LOG ENTEROCOCCUS	3.48	2.91	2.99	2.91	3.01	3.56	3.18	3.05	4.26	4.94	3.81	3.462
LOG FC	3.70	3.45	3.87	3.20	4.04	4.00	4.00	4.28	5.15	4.90	3.87	4.042
LOG TC	4.54		4.88	4.48	4.71	4.85	5.04	4.90	5.73	5.49	5.18	4.980
ENTEROCOCCUS	3000	820	980	820	1020	3600	1500	1120	18000	87000	6400	2900
FC	5000	2800	7400	1600	11000	10000	10000	19200	140000	79000	7400	11004
TC	35000		76000	30000	51000	70000	110000	80000	540000	310000	150000	95477

Table 2 - San Juan Watershed Study Bacterial Concentrations

Watershed: *1) San Juan*

Station Number SJ04 ***Location:*** SAN JUAN CREEK D/S OF STONEHILL DRIVE ***Mile:*** 0.9

Indicator	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8	Week 9	Week 10	Week 11	Mean (Log)
LOG ENTEROCOCCUS	2.00	2.04	3.18	2.38	2.38	2.30	1.70	2.11	2.20	1.95	2.15	2.218
LOG FC	2.46	1.60	2.86	2.15	2.45	1.60	1.60	2.30	2.26	2.00	2.34	2.147
LOG TC	3.64	3.00	3.48	3.20	3.04	2.90	4.07	4.11	3.72	3.62	3.70	3.499
ENTEROCOCCUS	100	110	1500	240	240	200	50	130	160	90	140	165
FC	290	40	720	140	280	40	40	200	180	100	220	140
TC	4400	1000	3000	1600	1100	800	11800	13000	5200	4200	5000	3158

Station Number SJ26 ***Location:*** SAN JUAN CREEK D/S OF TRABUCO ***Mile:*** 2.3

Indicator	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8	Week 9	Week 10	Week 11	Mean (Log)
LOG ENTEROCOCCUS	2.81	2.23	2.51	2.15	2.68	2.30	2.26	1.70	1.60	1.60	2.20	2.185
LOG FC	2.38	2.00	2.11	2.20	2.54	2.38	1.95	2.26	2.08	2.38	2.62	2.265
LOG TC	3.88	3.07	3.79	3.79	3.82	3.66	3.86	3.48	4.04	3.90	3.78	3.734
ENTEROCOCCUS	640	170	320	140	480	200	180	50	40	40	160	153
FC	240	100	130	160	350	240	90	180	120	240	420	184
TC	7600	1180	6200	6200	6600	4600	7200	3000	11000	8000	6000	5423

Table 2 - San Juan Watershed Study Bacterial Concentrations

Watershed: *1) San Juan*

Station Number SJ06 ***Location:*** SAN JUAN CREEK U/S OF TRABUCO ***Mile:*** 2.4

Indicator	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8	Week 9	Week 10	Week 11	Mean (Log)
LOG ENTEROCOCCUS	2.30	2.20	2.52	2.26	2.00	2.00	1.60	1.60	1.30	1.30	1.70	1.889
LOG FC	2.26	2.08	2.20	2.00	1.78	1.30	2.08	2.00	2.15	1.90	1.85	1.963
LOG TC	3.90	3.62	4.03	3.93	3.98	4.32	4.53	3.00	4.15	4.18	3.60	3.932
ENTEROCOCCUS	200	160	330	180	100	100	40	40	20	20	50	78
FC	180	120	160	100	60	20	120	100	140	80	70	92
TC	8000	4200	10800	8600	9600	21000	34000	1000	14000	15000	4000	8555

Station Number SJ07 ***Location:*** L01S09 AT LA NOVIA ***Mile:*** 3.7

Indicator	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8	Week 9	Week 10	Week 11	Mean (Log)
LOG ENTEROCOCCUS	3.51	2.45	3.98	3.66	2.89	4.78	2.62	3.41	3.60	3.75	3.91	3.507
LOG FC	2.85	2.15	3.30	3.00	3.23	3.86	2.45	2.78	3.53	3.38	4.09	3.146
LOG TC	3.93	3.56	4.30	3.88	3.62	5.04	4.11	4.95	4.61	4.78	4.95	4.341
ENTEROCOCCUS	3220	280	9600	4600	780	60000	420	2600	4000	5600	8200	3211
FC	700	140	2000	1000	1700	7200	280	600	3400	2400	12400	1401
TC	8600	3600	20000	7600	4200	110000	13000	90000	41000	60000	90000	21926

Table 2 - San Juan Watershed Study Bacterial Concentrations

Watershed: *1) San Juan*

Station Number SJ08 ***Location:*** SAN JUAN CREEK U/S OF LA NOVIA ***Mile:*** 3.71

Indicator	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8	Week 9	Week 10	Week 11	Mean (Log)
LOG ENTEROCOCCUS	2.58	1.90	2.04	2.70	2.56	2.70	2.76	2.30	2.70	1.78	3.04	2.460
LOG FC		2.08	2.34	1.78	2.30	2.38	3.13	2.38	2.00	1.30	3.08	2.277
LOG TC	4.11	3.81	5.04	4.00	3.70	3.98	3.68	3.48	4.40	4.76	5.00	4.178
ENTEROCOCCUS	380	80	110	500	360	500	580	200	500	60	1100	288
FC		120	220	60	200	240	1360	240	100	20	1200	189
TC	13000	6400	110000	10000	5000	9600	4800	3000	25000	57000	100000	15056

Station Number SJ09 ***Location:*** SAN JUAN D/S OF ORTEGA HWY ***Mile:*** 5.8

Indicator	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8	Week 9	Week 10	Week 11	Mean (Log)
LOG ENTEROCOCCUS	2.68	2.62	2.64	3.25	3.06	3.03	3.26	3.86	3.08	3.90	2.64	3.092
LOG FC	2.30	2.48	2.58	2.64	2.49	2.58	2.78	3.78	3.05	3.34	2.40	2.765
LOG TC	3.30	4.32	4.00	4.61	4.08	4.00	4.56	4.30	4.74	4.70	4.69	4.300
ENTEROCOCCUS	480	420	440	1760	1140	1060	1800	7200	1200	8000	440	1236
FC	200	300	380	440	310	380	600	6000	1120	2200	250	583
TC	2000	21000	10000	41000	12000	10000	36000	20000	55000	50000	49000	19961

Table 2 - San Juan Watershed Study Bacterial Concentrations

Watershed: *1) San Juan*

Station Number SJ30 ***Location:*** SAN JUAN U/S OF ORTEGA HWY ***Mile:*** 5.81

Indicator	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8	Week 9	Week 10	Week 11	Mean (Log)
LOG ENTEROCOCCUS			2.75	3.06	3.07	3.07	2.85	2.89	2.76	2.93	2.75	2.904
LOG FC			2.30	2.53	2.45	2.49	2.34	2.38	2.83	2.46	2.40	2.465
LOG TC			4.19		4.18	4.30	3.64	4.43	4.62	4.51	4.84	4.339
ENTEROCOCCUS			560	1140	1180	1180	700	780	580	860	560	801
FC			200	340	280	310	220	240	680	290	250	292
TC			15600		15000	20000	4400	27000	42000	32000	69000	21829

Table 2 - San Juan Watershed Study Bacterial Concentrations

Watershed: *2) Trabuco*

Station Number SJ05 ***Location:*** TRABUCO CREEK U/S OF SAN JUAN ***Mile:*** 0

Indicator	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8	Week 9	Week 10	Week 11	Mean (Log)
LOG ENTEROCOCCUS	3.37	1.78	2.58	2.60	2.82	2.79	2.30	2.08	2.20	2.26	2.62	2.491
LOG FC	2.79	1.78	3.66	2.93	2.60	2.40	2.75	2.18	2.38	2.83	2.30	2.601
LOG TC	3.53		3.66	4.18	3.66	3.51	3.53	3.60	3.30	3.48	3.85	3.630
ENTEROCOCCUS	2330	60	380	400	660	620	200	120	160	180	420	310
FC	620	60	4600	860	400	250	560	150	240	680	200	399
TC	3400		4600	15000	4600	3200	3400	4000	2000	3000	7000	4261

Station Number SJ31 ***Location:*** TRABUCO CREEK AT PASEO ADELANTO & RAMOS ST. ***Mile:*** 0.7

Indicator	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8	Week 9	Week 10	Week 11	Mean (Log)
LOG ENTEROCOCCUS		2.15	1.78	2.38	2.66	2.87	2.88	2.34	2.78	2.26	2.90	2.500
LOG FC		2.53	2.08	2.60	2.78	2.68	2.70	2.91	3.15	2.46	3.44	2.733
LOG TC		3.00	3.00	3.41	3.53	3.51	3.70	3.48	4.60	4.30	4.38	3.691
ENTEROCOCCUS		140	60	240	460	740	760	220	600	180	800	316
FC		340	120	400	600	480	500	820	1400	290	2740	541
TC		1000	1000	2600	3400	3200	5000	3000	40000	20000	24000	4910

Table 2 - San Juan Watershed Study Bacterial Concentrations

Watershed: *2) Trabuco*

Station Number SJ11 ***Location:*** L02P02 AT TRABUCO ***Mile:*** 0.8

Indicator	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8	Week 9	Week 10	Week 11	Mean (Log)
LOG ENTEROCOCCUS	4.05	4.37	4.18	4.71	5.45					3.94	3.81	4.358
LOG FC	4.43	4.18	3.81	5.54	4.23					3.15	4.08	4.202
LOG TC	6.16		7.17	6.52	4.72					5.54	5.00	5.852
ENTEROCOCCUS	11200	23600	15200	51000	280000					8800	6400	22829
FC	27000	15000	6400	350000	17000					1400	12000	15919
TC	1440000		4900000	3300000	52000					350000	100000	710704

Station Number SJ10 ***Location:*** TRABUCO CREEK AT RAMOS ST. U/S OF L02P02 ***Mile:*** 0.9

Indicator	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8	Week 9	Week 10	Week 11	Mean (Log)
LOG ENTEROCOCCUS	2.08	2.26	2.87	2.65	2.60	3.04	2.89	2.78	2.93	2.38	3.28	2.706
LOG FC	2.15	2.61	2.94	3.26	2.51	2.76	2.68	2.89	3.08	2.78	3.60	2.842
LOG TC	2.81	2.98	3.68	3.26	3.34	3.56	4.15	3.70	5.08	3.60	4.36	3.683
ENTEROCOCCUS	120	180	740	450	400	1100	780	600	860	240	1900	508
FC	140	410	880	1800	320	580	480	780	1200	600	4000	695
TC	640	960	4800	1800	2200	3600	14000	5000	120000	4000	23000	4818

Table 2 - San Juan Watershed Study Bacterial Concentrations

Watershed: *2) Trabuco*

Station Number SJ27 ***Location:*** TRABUCO CREEK D/S OF OSO CREEK ***Mile:*** 2.1

Indicator	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8	Week 9	Week 10	Week 11	Mean (Log)
LOG ENTEROCOCCUS	1.78	1.90	2.60	1.60	2.45	2.30	2.15	2.41	1.78	2.44	2.43	2.167
LOG FC	1.60	2.30	2.34	2.26	2.41	2.72	1.90	2.00	2.64	3.08	2.38	2.331
LOG TC	3.41	3.15	3.38	3.15	3.19	3.62	3.20	3.70	4.11	6.78	4.23	3.812
ENTEROCOCCUS	60	80	400	40	280	200	140	260	60	273	270	147
FC	40	200	220	180	260	520	80	100	440	1200	240	214
TC	2600	1400	2400	1400	1560	4200	1600	5000	13000	6000000	17000	6483

Station Number SJ12 ***Location:*** TRABUCO CREEK U/S OF OSO CREEK ***Mile:*** 2.2

Indicator	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8	Week 9	Week 10	Week 11	Mean (Log)
LOG ENTEROCOCCUS	1.60	1.90	2.08	1.90	2.53	2.45	2.20	2.18	2.08	2.52	2.26	2.154
LOG FC	1.60	1.90	1.70	2.15	2.30	2.49	1.90	1.48	2.08	4.00	2.64	2.204
LOG TC	3.45	3.01	2.60	3.48	2.83	3.41	3.78	3.38	4.54	6.70	4.36	3.777
ENTEROCOCCUS	40	80	120	80	340	280	160	150	120	330	180	143
FC	40	80	50	140	200	310	80	30	120	10000	440	160
TC	2800	1020	400	3000	680	2600	6000	2400	35000	5000000	23000	5982

Table 2 - San Juan Watershed Study Bacterial Concentrations

Watershed: *2) Trabuco*

Station Number SJ24 ***Location:*** TRABUCO CREEK AT CAMINO CAPISTRANO ***Mile:*** 2.7

Indicator	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8	Week 9	Week 10	Week 11	Mean (Log)
LOG ENTEROCOCCUS	2.08	2.15	2.48	2.41	2.41	2.56	2.62	2.54	2.41	2.20	2.92	2.436
LOG FC	2.18	1.90	2.20	2.53	2.38	2.90	2.62	2.79	2.64	2.72	2.79	2.515
LOG TC	3.48	3.30	3.26	3.41	3.32	3.58	3.53	3.90	3.81	3.64	3.90	3.558
ENTEROCOCCUS	120	140	300	260	260	360	420	350	260	160	840	273
FC	150	80	160	340	240	800	420	620	440	520	620	327
TC	3000	2000	1800	2600	2100	3800	3400	8000	6400	4400	8000	3614

Station Number SJ16 ***Location:*** TRABUCO CREEK AT SANTA MARGARITA PARKWAY ***Mile:*** 12.2

Indicator	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8	Week 9	Week 10	Week 11	Mean (Log)
LOG ENTEROCOCCUS	2.83	2.72	2.62	2.83	3.04	2.68	2.20	3.00	2.60	3.16	3.01	2.791
LOG FC	2.85	2.43	1.30	2.00	3.04	2.36	2.58	2.52	2.70	2.73	3.24	2.523
LOG TC	3.30	2.79	3.34	2.88	3.66	4.11	3.30	3.70	3.66	3.70	2.30	3.341
ENTEROCOCCUS	680	520	420	680	1100	480	160	1000	400	1440	1020	618
FC	700	270	20	100	1100	230	380	330	500	540	1745	333
TC	2000	620	2200	760	4600	13000	2000	5000	4600	5000	200	2195

Table 2 - San Juan Watershed Study Bacterial Concentrations

Watershed: *2) Trabuco*

Station Number SJ29 ***Location:*** TRABUCO CREEK D/S OF S19, WEST ***Mile:*** 14.4

Indicator	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8	Week 9	Week 10	Week 11	Mean (Log)
LOG ENTEROCOCCUS	1.30		2.66									1.982
LOG FC			2.38									2.380
LOG TC	2.60		5.26									3.929
ENTEROCOCCUS	20		460									96
FC			240									240
TC	400		180000									8485

Station Number SJ17 ***Location:*** TRABUCO CREEK U/S OF S19, EAST ***Mile:*** 14.5

Indicator	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8	Week 9	Week 10	Week 11	Mean (Log)
LOG ENTEROCOCCUS	2.00											2.000
LOG FC	2.94											2.944
LOG TC	3.04											3.041
ENTEROCOCCUS	100											100
FC	880											880
TC	1100											1100

Table 2 - San Juan Watershed Study Bacterial Concentrations

Watershed: *3) Oso*

Station Number SJ13 ***Location:*** OSO CREEK U/S OF TRABUCO ***Mile:*** 0

Indicator	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8	Week 9	Week 10	Week 11	Mean (Log)
LOG ENTEROCOCCUS	2.20	2.48	2.66	2.26	2.64	2.49	2.04	2.20	1.90	2.21	2.15	2.295
LOG FC	1.85	2.56	2.73	2.15	2.49	2.76	1.85	2.08	2.64	1.70	1.78	2.235
LOG TC	3.53	3.64	3.73	3.38	3.18	3.51	3.41	4.18	4.43	3.02	4.20	3.656
ENTEROCOCCUS	160	300	460	180	440	310	110	160	80	164	140	197
FC	70	360	540	140	310	580	70	120	440	50	60	172
TC	3400	4400	5400	2400	1520	3200	2600	15000	27000	1040	16000	4531

Station Number SJ14 ***Location:*** OSO CREEK AT THE END OF FORBES ROAD ***Mile:*** 3.3

Indicator	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8	Week 9	Week 10	Week 11	Mean (Log)
LOG ENTEROCOCCUS	2.38	2.08	2.26	3.34	3.32	4.70	4.52	2.58	3.85	2.46	2.51	3.090
LOG FC	2.54	2.20	2.70	3.45	3.87	5.04	5.26	3.20	3.48	3.11	3.00	3.441
LOG TC	3.34		4.08	4.04	3.97	5.93	6.00	4.48	4.70	4.48	4.70	4.572
ENTEROCOCCUS	240	120	180	2200	2070	50000	33000	380	7000	290	327	1231
FC	350	160	500	2800	7400	110000	180000	1600	3000	1300	1000	2763
TC	2200		12000	11000	9400	850000	1000000	30000	50000	30000	50000	37305

Table 2 - San Juan Watershed Study Bacterial Concentrations

Watershed: *3) Oso*

Station Number SJ19 ***Location:*** VIA ANGELINA STORM DRAIN AT OSO CREEK ***Mile:*** 5.3

Indicator	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8	Week 9	Week 10	Week 11	Mean (Log)
LOG ENTEROCOCCUS	2.26	3.92	2.54									2.908
LOG FC	2.00		3.03									2.517
LOG TC	3.41	4.78	3.85									4.013
ENTEROCOCCUS	180	8400	350									809
FC	100		1080									329
TC	2600	60000	7000									10298

Station Number SJ18 ***Location:*** OSO CREEK AT VIA CASITAS ***Mile:*** 5.4

Indicator	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8	Week 9	Week 10	Week 11	Mean (Log)
LOG ENTEROCOCCUS	2.46	1.95	2.73	2.73	2.64	2.45	2.51	2.53	3.11	3.06	3.13	2.666
LOG FC	2.34	2.66	3.18	2.89	2.49	2.51	2.64	2.92	3.30	3.20	3.58	2.884
LOG TC	3.46	3.60	3.90	3.72	3.57	3.73	3.90	3.90	4.54	4.36	4.57	3.933
ENTEROCOCCUS	290	90	540	540	440	280	320	340	1300	1160	1360	463
FC	220	460	1510	780	310	320	440	840	2000	1600	3800	766
TC	2900	4000	8000	5200	3700	5400	8000	8000	35000	23000	37000	8573

Table 2 - San Juan Watershed Study Bacterial Concentrations

Watershed: *3) Oso*

Station Number SJ21 ***Location:*** OSO CREEK AT DURANZO ***Mile:*** 7

Indicator	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8	Week 9	Week 10	Week 11	Mean (Log)
LOG ENTEROCOCCUS	3.08	2.40	2.73	2.65	2.72	2.43	2.30	2.93	2.90	3.56	3.38	2.826
LOG FC	2.75	3.64	2.75	2.70	2.46	2.40	2.00	2.75	2.79	2.90	3.53	2.789
LOG TC	3.76	2.98	3.95	4.34	3.79	4.76	3.48	4.30	3.85	3.64	4.73	3.963
ENTEROCOCCUS	1200	250	540	450	520	270	200	860	800	3600	2400	670
FC	560	4400	560	500	290	250	100	560	620	800	3400	615
TC	5800	960	9000	22000	6200	58000	3000	20000	7000	4400	54000	9192

Station Number SJ20 ***Location:*** L03P14 AT OSO CREEK ***Mile:*** 7.1

Indicator	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8	Week 9	Week 10	Week 11	Mean (Log)
LOG ENTEROCOCCUS	2.49	3.23	3.47	3.60	3.28	3.64	2.94	3.51	3.11	3.35	3.41	3.277
LOG FC	2.00		3.62	3.51	3.51	2.79	2.89	3.08	3.62	3.51	3.41	3.194
LOG TC	3.56	4.30	5.00	4.90	4.18	4.30	3.85	4.85	4.46	4.52	4.48	4.399
ENTEROCOCCUS	310	1700	2980	4000	1900	4400	880	3200	1300	2240	2600	1893
FC	100		4200	3200	3200	620	780	1200	4200	3200	2600	1563
TC	3600	20000	100000	80000	15000	20000	7000	70000	29000	33000	30000	25044

Table 2 - San Juan Watershed Study Bacterial Concentrations

Watershed: *3) Oso*

Station Number SJ32 ***Location:*** OSO CREEK AT OLYMPAID D/S OF BRANCHES (L03P18) ***Mile:*** 9.99

Indicator	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8	Week 9	Week 10	Week 11	Mean (Log)
LOG ENTEROCOCCUS	4.48	3.79	3.83	3.57	4.20	3.38	3.90	3.22	4.90	3.88	4.18	3.940
LOG FC	4.48	3.15	3.53	3.38	3.26	3.30	3.58	3.78	4.65	3.98		3.708
LOG TC	5.46	4.60	4.48	3.86	4.34	5.15	4.78	4.90	5.67	5.40	5.49	4.921
ENTEROCOCCUS	30000	6200	6800	3690	16000	2400	8000	1650	80000	7600	15200	8709
FC	30000	1400	3400	2400	1800	2000	3800	6000	45000	9600		5111
TC	290000	40000	30000	7200	22000	140000	60000	80000	470000	250000	310000	83352

Station Number SJ23 ***Location:*** L03P20 & L03P29 AT OSO CREEK ***Mile:*** 10

Indicator	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8	Week 9	Week 10	Week 11	Mean (Log)
LOG ENTEROCOCCUS	4.23	4.10	4.35	4.79	4.20	3.41	4.51	4.48	3.98	4.26	4.30	4.237
LOG FC	4.15	3.56	3.51	4.30	3.79	3.56	4.26	5.00	4.29	4.04	4.60	4.095
LOG TC	3.38	4.78	4.48	4.22	4.70	5.11	5.00	5.82	4.70	5.30	5.62	4.829
ENTEROCOCCUS	17000	12600	22200	62000	16000	2600	32000	30000	9600	18000	20000	17267
FC	14000	3600	3200	20000	6200	3600	18000	100000	19300	11000	40000	12436
TC	2400	60000	30000	16700	50000	130000	100000	660000	50000	200000	420000	67381

Table 2 - San Juan Watershed Study Bacterial Concentrations

Watershed: *3) Oso*

Station Number SJ28 ***Location:*** WEST DRAINAGE ENTERING OSO CREEK AT OLYMPIAD ***Mile:*** 10

Indicator	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8	Week 9	Week 10	Week 11	Mean (Log)
LOG ENTEROCOCCUS	3.33	4.61	3.99	3.30	3.90	4.21	3.48	3.83	4.84	4.18	3.15	3.894
LOG FC	2.60	3.73	3.73	3.40	3.41	2.68	3.00	2.34	4.21	3.38	3.58	3.280
LOG TC	5.00		4.60	4.04	4.51	4.00	4.28	3.18	5.49	4.43	4.60	4.413
ENTEROCOCCUS	2150	40400	9800	2000	8000	16400	3000	6800	69000	15200	1420	7830
FC	400	5400	5400	2510	2600	480	1000	220	16400	2400	3800	1905
TC	100000		40000	11000	32000	10000	19000	1500	310000	27000	40000	25872

Station Number SJ22 ***Location:*** OSO CREEK AT OLYMPIAD ABOVE BRANCHES ***Mile:*** 10.0

Indicator	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8	Week 9	Week 10	Week 11	Mean (Log)
LOG ENTEROCOCCUS	2.00	2.43	2.15	2.88	3.18	2.88	3.30	2.94	4.90	3.21	3.78	3.059
LOG FC	2.97	3.26	3.73	3.18	3.18	3.41	3.37	3.62	4.34	4.04	4.24	3.576
LOG TC	3.92	4.60	4.38	3.88	4.48	4.48	4.30	4.48	5.48	4.60	5.34	4.540
ENTEROCOCCUS	100	270	140	760	1500	760	2000	880	79000	1640	6000	1146
FC	940	1800	5400	1500	1500	2600	2360	4200	21800	11000	17200	3770
TC	8400	40000	24000	7600	30000	30000	20000	30000	300000	40000	220000	34684

Table 2 - San Juan Watershed Study Bacterial Concentrations

Watershed: *4) Wagon Wheel*

Station Number SJ15 ***Location:*** WAGON WHEEL CREEK U/S OF CROSSING IN RILEY PARK ***Mile:*** 1.2

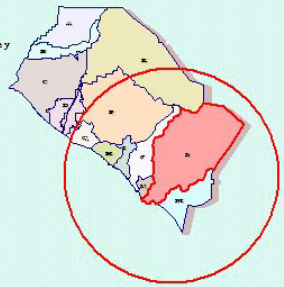
Indicator	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8	Week 9	Week 10	Week 11	Mean (Log)
LOG ENTEROCOCCUS	2.78	3.26	2.96	2.90	4.20	3.19	3.62	3.28	3.18	3.41	3.46	3.296
LOG FC	2.79	2.83	2.91	2.38	3.08	2.36	3.11	2.85	2.87	2.81	3.26	2.841
LOG TC	3.75	3.56	3.85	3.30	4.08	3.45	4.11	4.26	4.34	4.67	4.67	4.003
ENTEROCOCCUS	600	1840	920	800	16000	1560	4200	1900	1500	2600	2870	1978
FC	620	680	820	240	1200	230	1300	700	740	640	1800	693
TC	5600	3600	7000	2000	12000	2800	13000	18000	22000	47000	47000	10069

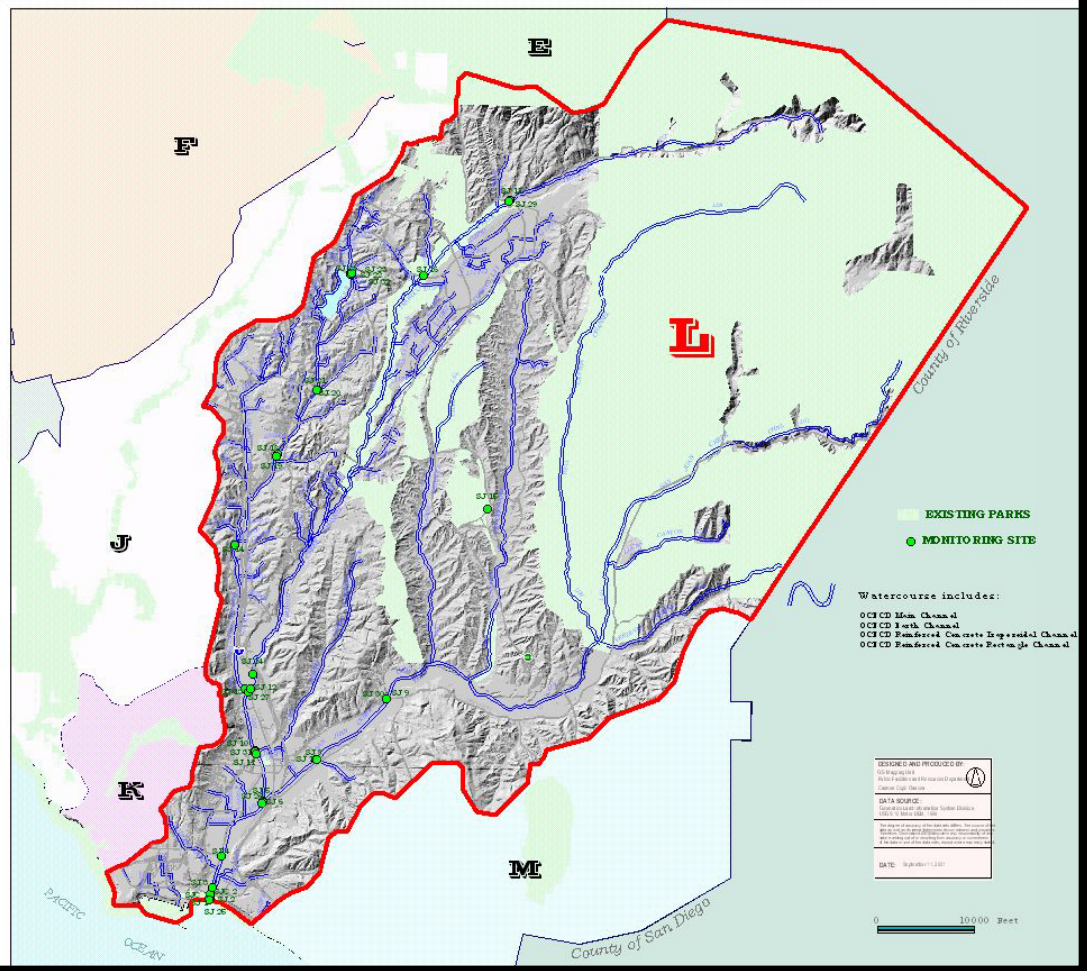
WATERSHED L : SAN JUAN CREEK

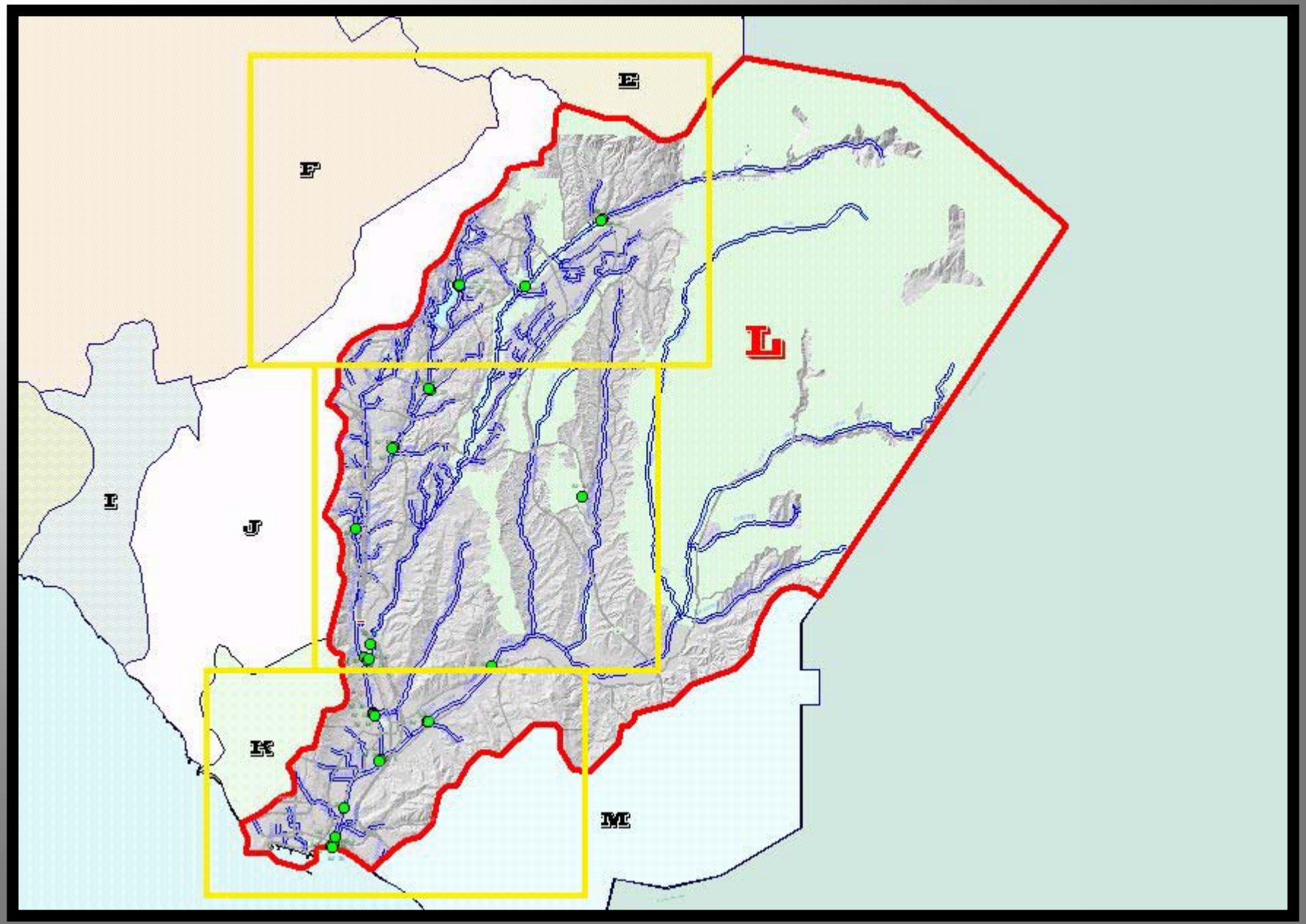
MONITORING SITES

COUNTY OF ORANGE, CALIFORNIA

SJ1 San Juan Creek at Beach (West)
SJC1 Lower San Juan Creek
SJ2 San Juan Creek at Beach (East)
SJC2 Upper San Juan Creek
SJ3 L01S02 at San Juan Creek
SJ4 San Juan Creek d/s of Stonehill
SJ5 Trabuco Creek u/s of San Juan
SJ6 San Juan Creek u/s of Trabuco
SJ7 L01S09 at La Novia
SJ8 San Juan Creek u/s of La Novia
SJ9 San Juan Creek d/s of Ortega Hwy
SJ10 Trabuco Creek at Ramos St. u/s of L02P02
SJ11 L02P02 at Trabuco
SJ12 Trabuco Creek u/s of Oso Creek
SJ13 Oso Creek u/s of Trabuco
SJ14 Oso Creek at end of Forbes Road
SJ15 Wagon Wheel Creek u/s of Crossing in Riley
SJ16 Trabuco creek at Santa Margarita Pkwy
SJ17 Trabuco Creek u/s of S19 (East)
SJ18 Oso Creek at Via Cazitas
SJ19 Via Angelina Storm Drain at Oso Creek
SJ20 L03P14 at Oso Creek
SJ21 Oso Creek at Duranzo
SJ22 Oso Creek at Olympiad above branches
SJ23 L03P20 & L03P29 at Oso Creek
SJ24 Trabuco Creek at Camino Capistrano
SJ25 Ocean at San Juan Creek
SJ26 San Juan Creek d/s of Trabuco
SJ27 Trabuco Creek d/s of Oso Creek
SJ28 West drainage entering Oso Creek at Olympia
SJ29 Trabuco Creek d/s of S19 (West)
SJ30 San Juan Creek u/s of Ortega Hwy
SJ31 Trabuco Creek at Paseo Adelanto & Ramos St.
SJ32 L03P18 Oso Creek at Olympiad d/s of branche



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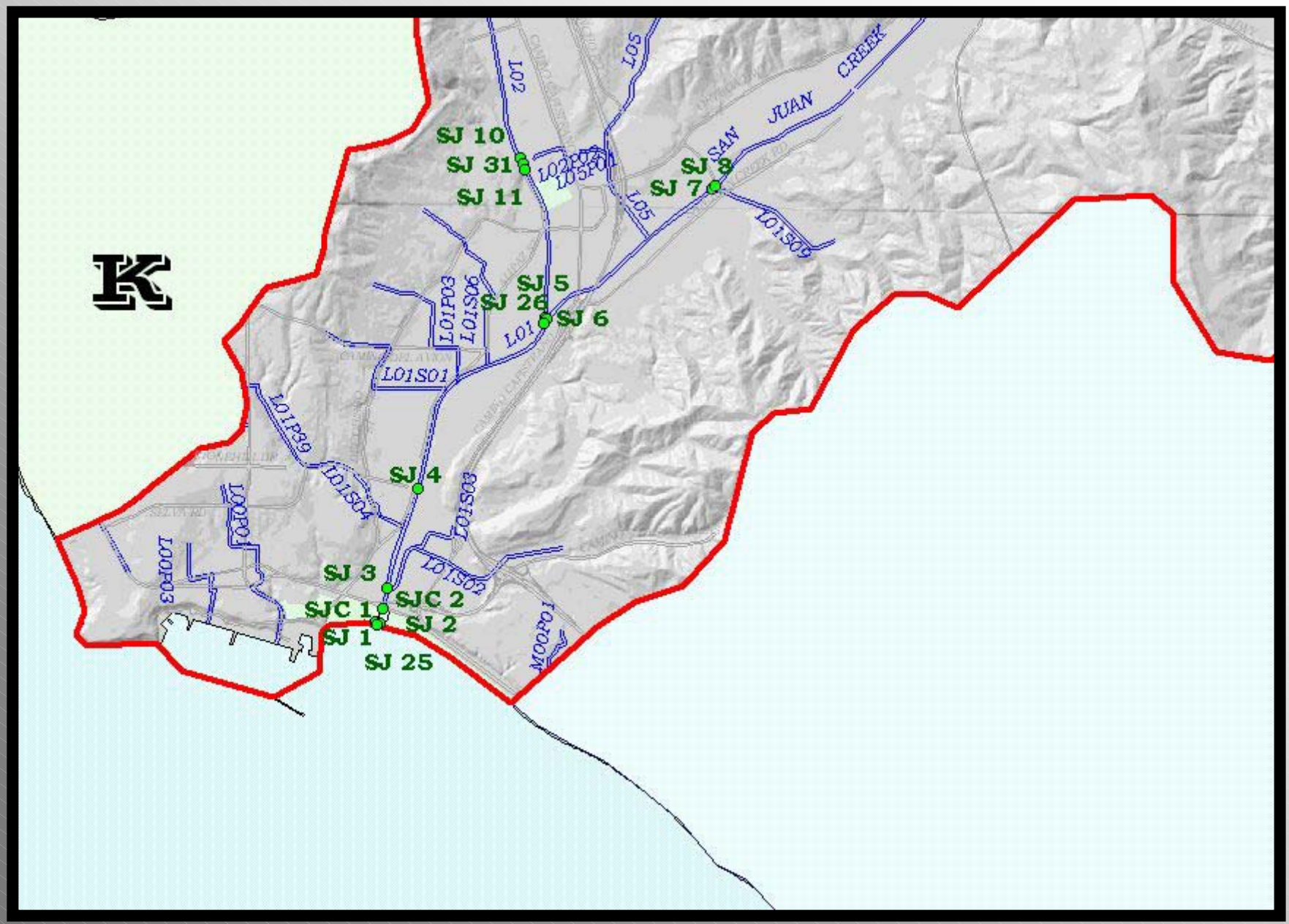
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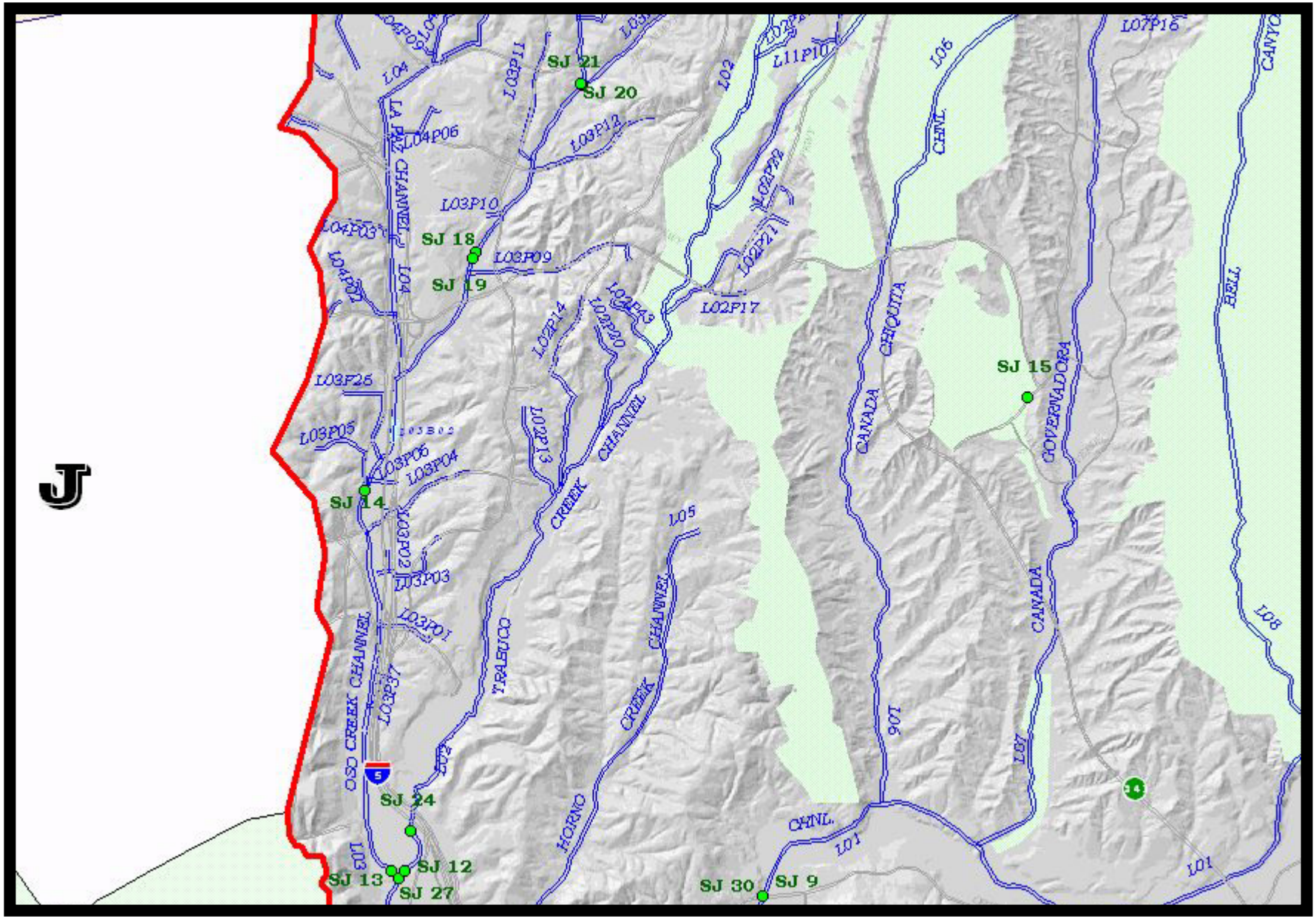
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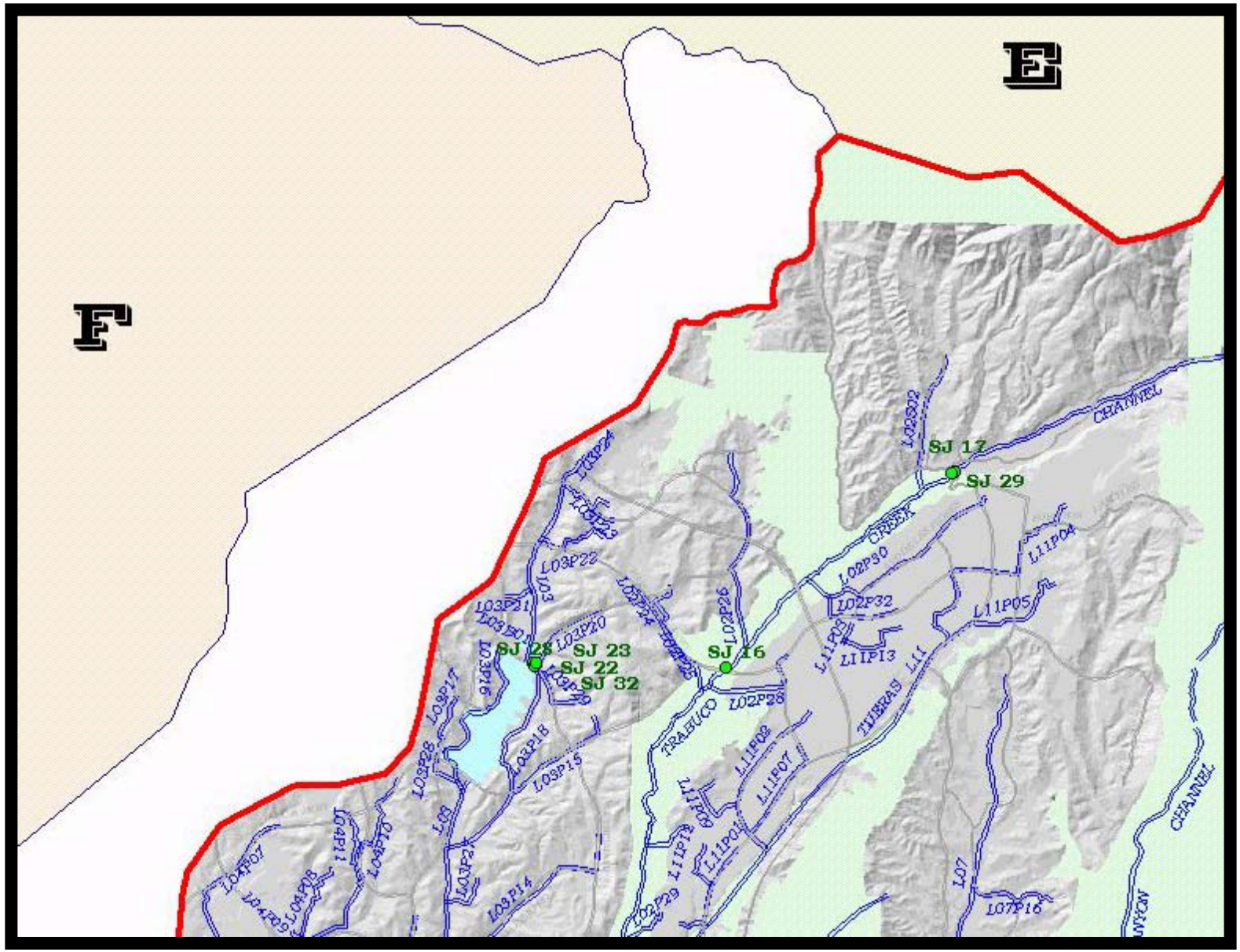
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CHAPTER 2

PHASE II: DETAILED BACTERIOLOGICAL SURVEY

OF

SAN JUAN CREEK WATERSHED

Contents

1. Introduction

During Phase I of the San Juan Creek (SJC) Watershed Bacteriological Study, water samples were collected from 36 sites from April 30 through July 10, 2001 to characterize the bacteriology of the watershed. The sites included 26 creek sites, 7 storm-drain sites and 3 ocean sites. The bacteriological results were used to select a small subset of locations for more detailed monitoring as part of Phase II. High levels of fecal coliform and *Enterococcus* were found at the lower SJC sampling sites where the creek flows into the Pacific Ocean. Possible reasons for high bacterial levels include contamination from intervening storm drain outlets, direct contamination from waterfowl or accumulation and potential survival of bacteria at the lower end of the creek. Therefore, 5 sites including 2 stations at the lower SJC, 1 ocean station and 2 creek stations upstream of the mouth of the San Juan Creek were monitored for 13 weeks to assess potential sources of bacterial contamination. None of the storm drains monitored during Phase I were sampled during Phase II. The 5 sampling sites are representative of different areas of the watershed affected by wild and domestic animals, storm drains, and potential human sources.

This report summarizes results for Phase II: bacteriological monitoring survey of problem areas identified in Phase 1 requiring further study.

2. Experimental Design/Sampling Plan

Samples were collected once a week for 13 weeks, beginning on September 19 through December 10, 2001 from 5 sites in the SJC watershed (Table 1). The sites included the following locations as depicted on the map (Figure 6):

(1) Pacific Ocean at the mouth of SJC (station number SJ25);

(2) East side of SJC at the mouth (SJ02) (behind berm);

(3) SJC below Pacific Coast Highway (PCH) (SJC2);

(4) SJC above Trabuco Creek (SJ06); and

(5) Trabuco Creek (SJ10).

SJ02 and SJC2 were identified as areas with high levels of bacterial contamination in Phase I. SJ02 is located behind a sand berm separating SJC from the Ocean. SJC2 is located in SJC at the PCH overpass about 0.2 miles from the ocean and 0.45 miles below storm drain L01S02, which had high fecal indicator levels during the Phase I study. SJ06 is located in SJC above the confluence of the San Juan and Trabuco Creeks, about 2.4 miles from the

ocean, and represents a more rural section of the watershed. SJ10 is located 0.9 miles up Trabuco Creek above the confluence of the San Juan and Trabuco Creeks and is the furthest sampling point from the ocean, and represents a suburban/urban watershed.

Water samples were tested for total and fecal coliforms, *Enterococcus*, and *E. coli* with Membrane Filtration media, methods and references specified as follows:

Total Coliform - MF/m-Endo - SM9222A & B
Fecal Coliform - MF/mFC - SM9222D
(Standard Methods for Examination of Water and Wastewater, 20th Edition, American Public Health Association, 1998)

Escherichia coli - MF/Modified m-Tec-USEPA Modified E. coli Method (1998)
Enterococci - MF/mEI- USEPA Method 1600
(EPA, Improved Enumeration Methods for the Recreational Water Quality Indicators: Enterococci and *Escherichia coli*, EPA/821/R-97/004; March 2000)

The bacterial concentrations are reported as colony forming units per 100 ml of water. The relative contribution of bacterial loading from each creek to the problem areas was compared. Bacterial levels obtained during Phase II were compared to Phase I sampling results to assess temporal differences.

There were two days during the 13-week sampling period when samples were collected during or one day following rain events. Rainfall data was obtained from PFRD Coastal and Water Resources using measurements taken at Santiago Peak.

In addition to determining fecal bacteria levels, *E. coli* and *Enterococcus* bacterial colonies were isolated from water samples collected at each location and frozen for further study (Phase III). One objective of Phase III is to conduct bacterial source identification testing in an effort to determine whether there are human and/or animal sources of bacteriological contamination, and to differentiate between specific fecal source categories (i.e. human, sewage, cat, dog, seagull and horse). Antibiotic Resistance Analysis (ARA) and ribotyping are two examples of source tracking tools recently reported as being useful in determining potential sources of specific bacterial strains.

3. Results

Bacterial levels in Watershed

The bacterial concentrations (log of the concentrations and the log mean of all samples) for each station by sampling week are presented in Table 2, “San Juan Creek Phase II Bacterial Concentrations”. A 5-point summary of bacterial concentrations by station are presented in Figures 1A-D. The points include the minimum value, 25th percentile, median, log mean, 75th

percentile and maximum value. Outliers, defined as values greater than 1.5 times the range between the 25th and 75th percentile, are also indicated.

The overall range of actual bacterial concentrations by sample type (creek vs. ocean) is presented in Table 3. Similar to Phase I, the bacterial concentrations for total and fecal coliforms, and *Enterococcus* were higher overall in San Juan and Trabuco Creeks as compared to levels detected in the Ocean (SJ25). *E. coli* levels (analyzed in Phase II only) were also higher in the creeks than the ocean. San Juan Creek sites below PCH (SJ02 and SJC2) had consistently high indicator levels during the Phase II study period, as was the case during Phase I. The highest log mean concentrations of total and fecal coliforms, *Enterococcus*, and *E. coli* were detected in samples from the mouth of SJC (SJ02) (Figure 1A-D).

Statistical Comparison of Phase I to Phase II

Statistical comparisons were analyzed for Phase I and Phase II (adjusting for the effect of rain). Data were $\log_{10}$ transformed before analysis to normalize the distributions and to reduce the influence of outliers and extreme values. Phase II data (including rain events) was first compared to Phase I results. For both studies, the highest bacterial concentrations were found in samples collected at the lower SJC area (SJ02 and SJC2), the areas targeted for Phase II research. Levels of fecal coliform, *Enterococcus* and *E. coli* were highest at sites SJ02 and SJC2. However, Phase II total coliform levels were highest at SJ02 and SJ10 (upstream).

By contrast, fecal coliform levels at SJC2 were higher than the upstream sites, suggesting possible high input from storm drain L01S02 and other storm drains. Whether increased bacterial levels at SJC2 are due to transport of bacteria from the storm drain was not determined during this study. Since SJ02 is located about 700 feet below SJC2, high indicator levels found here may be associated with high levels upstream at SJC2. This determination is complicated by the observation that the highest density of waterfowl is typically found year round at SJ02. SJ02 was also the location with the highest concentrations of *Enterococcus*.

During Phase II (omitting samples collected during rain events), geometric mean total coliform levels were about 2-4 times higher than Phase 1 levels at all 5 sites. In contrast, fecal coliform levels were approximately twice as high during Phase II, and *Enterococcus* were similar to Phase I levels except for stations SJ06 and SJ25 which were approximately 4 times higher in Phase II.

Comparison of Phase II to Phase I indicator levels reveal significantly higher concentrations in Phase II overall. However, on a station-by-station basis, there are relatively few significant differences. This is believed to be due to the small sample sizes (10 in Phase I versus 13-15 in Phase II for each station), and therefore minimal power to detect a statistically significant difference. However, significant increases in total coliforms from Phase I to Phase II were observed at sites SJ10 and SJ25. As for fecal coliforms, significant increases from Phase I to

Phase II were seen at sites SJ06, SJ10, and SJ25. *Enterococcus* levels were significantly higher in Phase II versus Phase I at sites SJ06 and SJ25.

Comparison of bacterial concentrations to basin plan water quality standards

The bacterial levels measured were compared to REC-1 (Contact recreation) and REC-2 (Non-contact recreation) standards defined in the Water Quality Objectives of the Basin Plan. The REC-1 standard states that "the fecal coliform concentration based on a minimum of not less than five samples for any 30-day period, shall not exceed a log mean of 200/100 ml nor shall more than 10 percent of total samples collected during a 30-day period exceed 400/100 ml." To apply this standard to the study results, which were taken over a period of only 13 weeks, the log mean was calculated for the weeks in which there were at least 4 previous results available. The results are presented in Figure 3 – "Compliance with REC-1 Standard". All 5 sites had sufficient results for this analysis; 3 creek sites (SJ02, SJC2, and SJ10) had zero% compliance with the standard. SJ25 had 14.3% compliance and SJ06 had 44.0% compliance.

The REC-2 standard states that "the average fecal coliform concentrations for any 30-day period, shall not exceed 2000/100ml nor shall more than 10 percent of samples collected during any 30-day period exceed 4000/100ml." To apply this standard to the study results, which were taken over a period of only 13 weeks, the average was calculated for the weeks in which there were at least 4 previous results available. The results are presented in Figure 4 – "Compliance with REC-2 Standard". All 5 sites had sufficient results for this analysis; sites ranged from 33% to 100% compliance with the standard.

Effect of Rain

Two rain events were seen during this phase of the study. The log mean concentrations comparing Phase I and Phase II results (Figures 2A-C) are segregated based on rain events. Phase II results "rain" are for samples collected during two rain events, November 13 and December 4 (following rainfall of December 3), with 0.47 and 1.06 inches of rainfall, respectively.

There were 0.24 to 1.50 log increases in levels of all fecal indicator concentrations in samples collected at all 5 sites during the rain events (Figure 2, A-C), with the exception of slightly lower total coliform levels at SJ06. Log mean differences in concentration levels for data including and excluding rain events are listed in Table 4. Of the 5 sites, the greatest increases in total and fecal coliform and *Enterococcus* levels during rain events were found at SJ02, SJC2 and SJ25. *E. coli* levels experienced the greatest increase with rain at SJ10, SJ25, and SJ02, respectively. Levels at SJ02 and SJC2 (SJC, below PCH) remained elevated for the duration of the study (4 weeks post rain events).

The increased bacterial levels at the ocean and creeks during Phase II are associated with rainfall (Figures 2A-D), and corresponding increases in urban runoff, and possibly with higher waterfowl density in the fall and winter as compared to summer.

Phase II data was then evaluated for the 11-week period excluding rain events (Table 4). This analysis revealed uniform levels of total coliform across creek samples (with lower levels at SJ25, ocean). Fecal coliform levels were uniformly higher across stations SJC2, SJ02 (creek sites), and SJ10 (upstream). *Enterococcus* and *E. coli* levels were highest at SJ02 and SJC2. It is also interesting to note that all stations showed considerable increases in indicator levels on November 5, 2001, which experienced "light rain" = 0.04 inches (insufficient to trigger a rain advisory).

Effect of Opening Berm

On November 13, the berm between the mouth of SJC and the ocean was opened due to flooding and warning signs prohibiting ocean water contact were posted at Doheny Beach (SJ25). Bacterial indicator levels from samples collected at the ocean were significantly higher than normal levels and reverted back to normal levels one week later.

The initial rain event required the berm to be opened and to remain open for the duration of the study (through December 10). Levels at the ocean were highest during the initial rain event with additional input of high concentrations of bacteria from SJC. Indicator levels at the ocean were at normal levels within one week of the first rain event, and within three weeks of the second event. The berm remained opened through the end of the sampling period, and the indicator levels at the lower SJC area remained high for the duration.

Comparison of Fecal Coliform to *E. coli*

Levels of fecal coliform relative to *E. coli* were analyzed and the ratios for both untransformed and $\log_{10}$ transformed data (13 weeks) are presented in Figure 5. The bivariate correlation between fecal coliform and *E. coli* ranged from 0.86 to 0.98 among the five stations; all correlations were significant at the 0.01 level. The similarity between concentrations of these indicators suggests human and/or animal sources, and not environmental sources.

The correlation was most pronounced at SJ02, with a dry weather correlation of 0.97. Overall, the ratio of fecal coliform to *E. coli* was slightly higher for the creek samples on the rain dates versus dry weather weeks. The greatest difference in the ratio for rain compared to dry weather was observed at station SJ25 (ocean), which had a correlation of 0.65 in dry weather and 0.95 during rain events. The creek station correlations ranged from 0.80 to 0.97 in dry weather, and from 0.95 to 1.00 with rain.

4. Conclusions

1. Bacterial pollution measured by levels of standard indicator organisms was ubiquitous in creek samples in both Phases I and II. Overall, stations at lower SJC (SJ02 and SJC2) had higher indicator concentrations than upstream or at the ocean. Excluding the effects of rain, Phase II levels remained higher overall than Phase I.

2. In both Phase I and II, mean concentrations of indicator organisms in creeks were similar to those impacted by urban runoff (Aliso watershed, unpublished reports). Mean levels indicative of large or moderate amounts of direct sewage were not seen in either phase. However, occasional spikes in indicator levels in some creek sites are suggestive of single direct contamination events.

3. Indicator levels were higher in creeks and the ocean following rain evens than during dry weather.

4. As in Phase I, creek sites had moderate concentrations of indicator organisms, ranging from a geometric mean fecal coliform of 245 at station SJ06 (SJC above Trabuco Creek) to 5,220 at station SJ02 (east side of the mouth of San Juan Creek behind berm).

5. In both phases, samples collected at the lower SJC area (SJ02 and SJC2), were observed to have the highest levels of fecal coliform, *Enterococcus* and *E. coli*. However, Phase II total coliform levels were highest at SJ02 and SJ10 (upstream). Among individual stations, there were significant increases in indicator levels from Phase I to Phase II for fecal coliform (SJ06, SJ10, and SJ25) and *Enterococcus* (SJ06 and SJ25).

6. In both phases, overall water quality measured against REC-1 standards was poor. In Phase II, 3 of the 5 sites had zero % compliance with the standard.

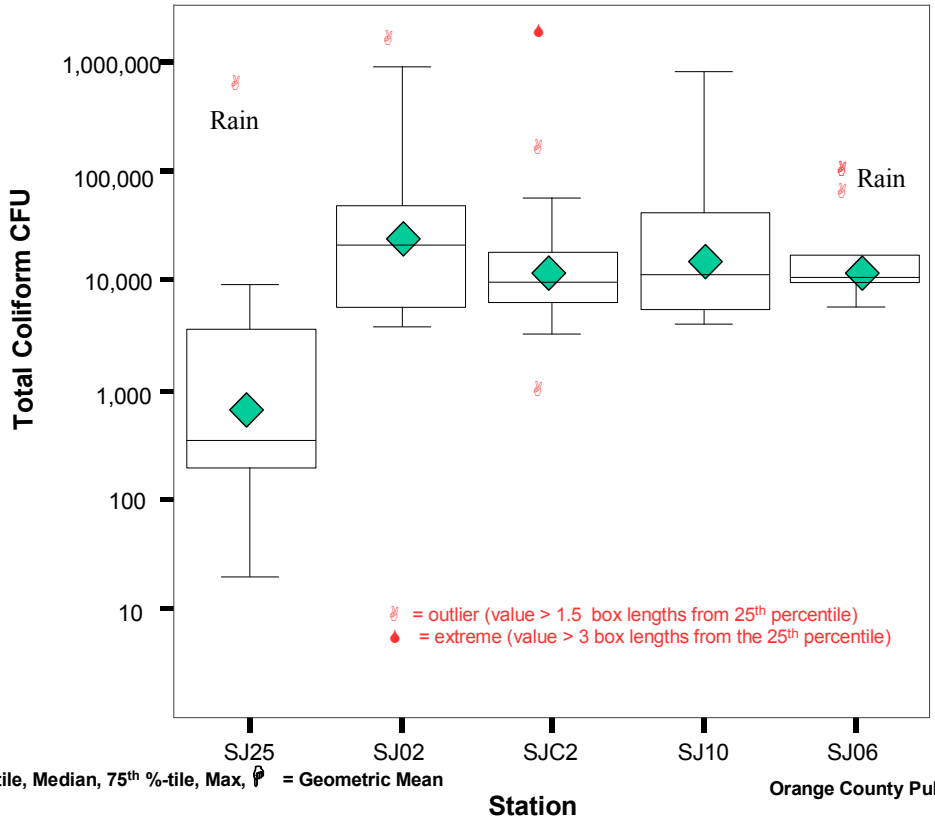
7. Adjusting for the effects of two rain events, geometric mean total coliform levels were about 2-4 times higher than Phase 1 levels at all 5 sites. Fecal coliform levels were approximately twice as high during Phase II, and *Enterococcus* were similar to Phase I levels except for stations SJ06 and SJ25 which were approximately 4 times higher in Phase II. Bacterial levels (excluding rain-influenced data) at the ocean and creeks during Phase II averaged ¼ to ½ log higher than Phase I, and may be associated with higher waterfowl density in the fall and winter as compared to summer.

8. The concentrations of *E. coli*, a more specific subset of animal/human fecal coliforms, and fecal coliforms were significantly correlated at all sites. The correlations ranged from 0.86 to 0.98 across the five sites. The highest correlation of fecal coliform and *E. coli* was at SJ02, indicating that the majority of fecal coliform organisms seen were *E. coli*, indicative of fecal contamination.

9. The ratio of fecal coliforms to *E. coli* concentrations varied moderately by sample. Fifty percent of samples had a ratio ranging from 1.0 to 3.5; the remainder were < 1.0 or > 2.5, indicating that a single “conversion factor” should not be used to convert *E. coli* contamination to equivalent fecal coliform contamination.

List of Figures

Figure 1A – San Juan Creek Watershed Phase II: 5-Point Summaries* and Means by Location Total Coliform



*Min, 25th %-tile, Median, 75th %-tile, Max, = Geometric Mean

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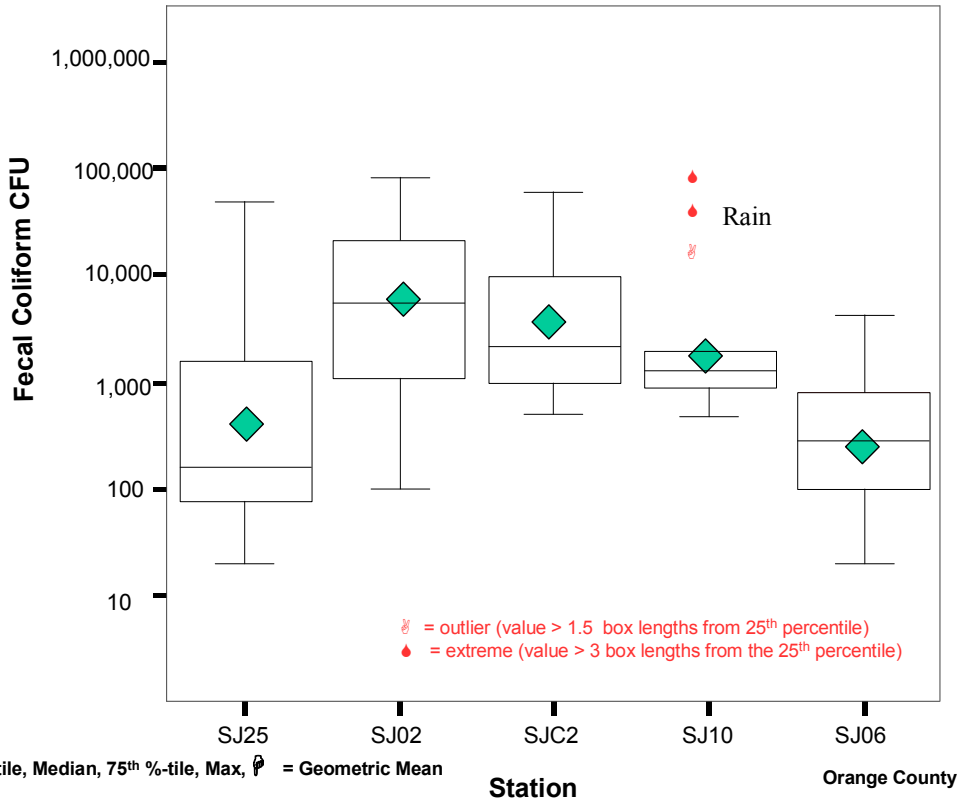
Figure 1B – San Juan Creek Watershed Phase II: 5-Point Summaries* and Means by Location Fecal Coliform



*Min, 25th %-tile, Median, 75th %-tile, Max, = Geometric Mean

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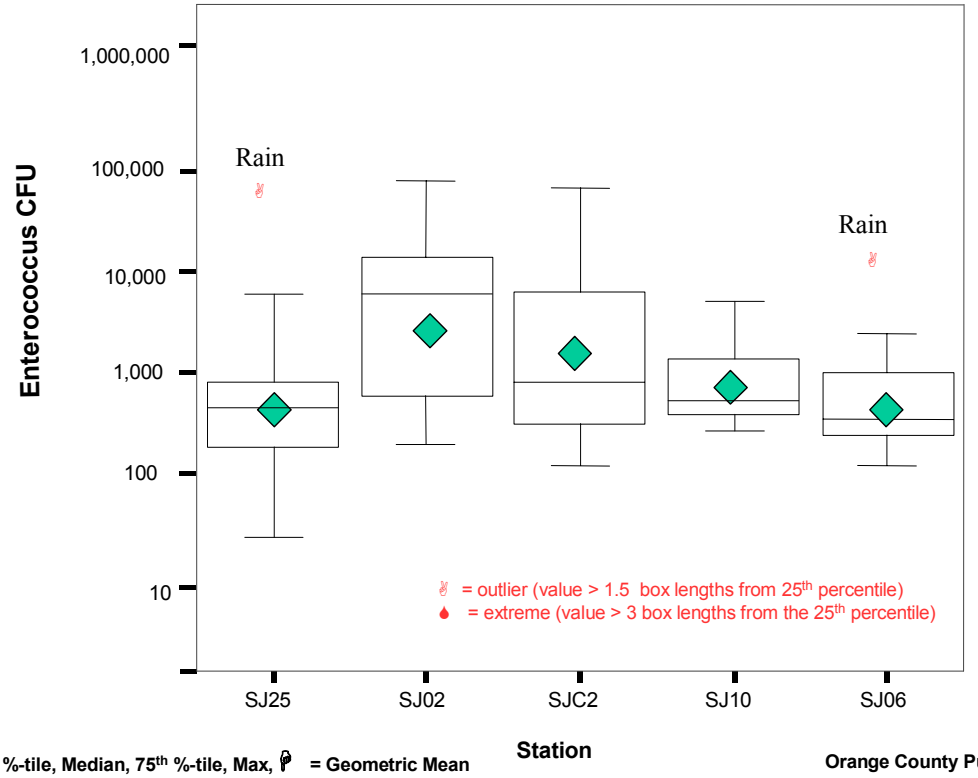
Figure 1C – San Juan Creek Watershed Phase II: 5-Point Summaries* and Means by Location Enterococcus



*Min, 25th %-tile, Median, 75th %-tile, Max, = Geometric Mean

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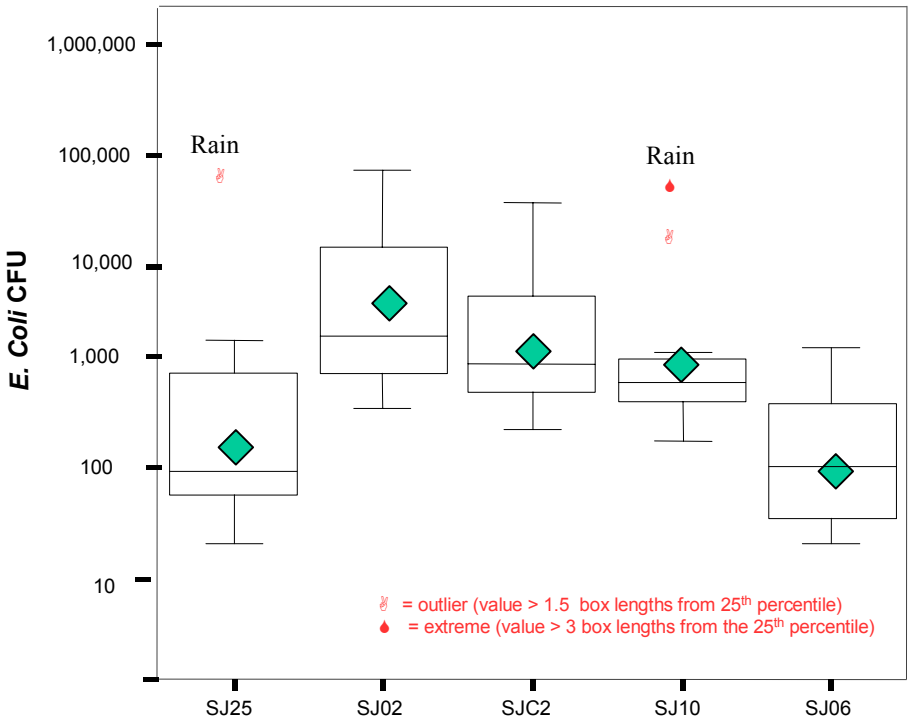
Figure 1D – San Juan Creek Watershed Phase II: 5-Point Summaries* and Means by Location

E. coli



*Min, 25th %-tile, Median, 75th %-tile, Max, = Geometric Mean

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Figure 2A -San Juan Watershed Total Coliform
Phase I (April – July) and
Phase II (September – December)

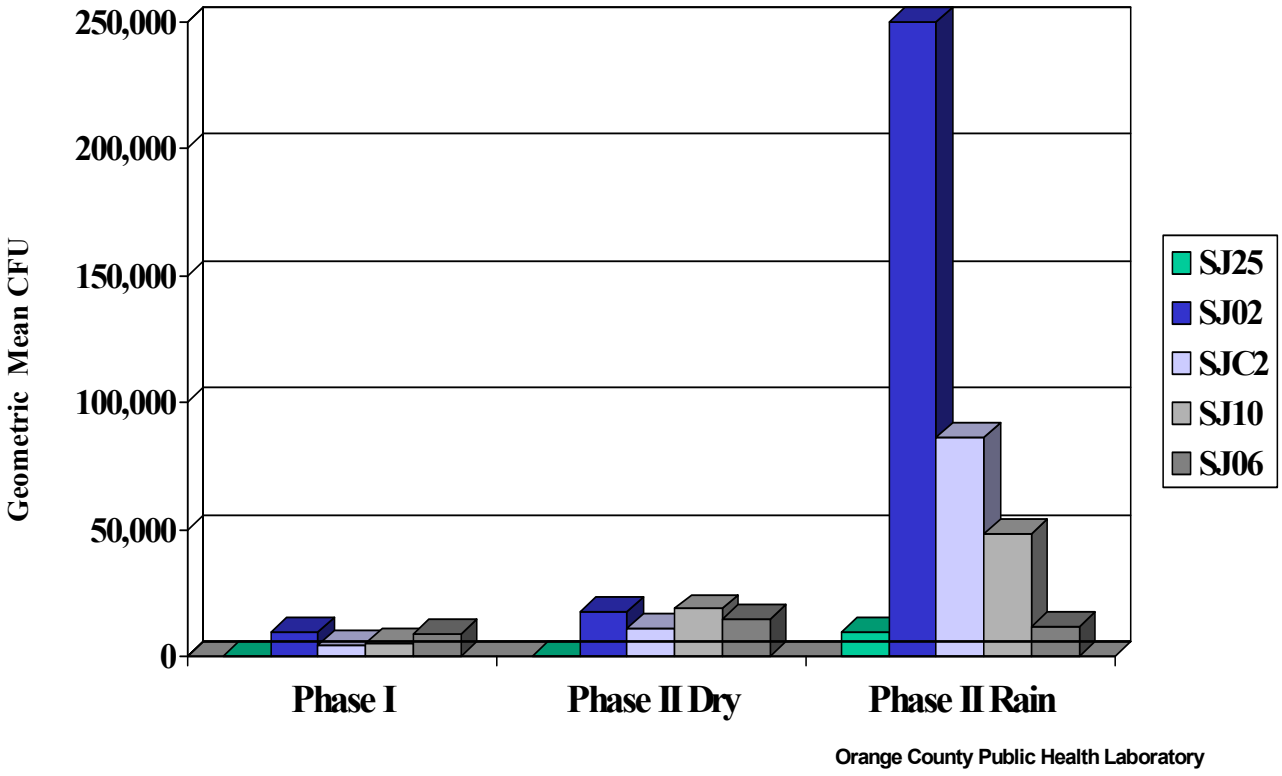

Figure 2B – San Juan Watershed Fecal Coliform
Phase I (April – July) and
Phase II (September – December)

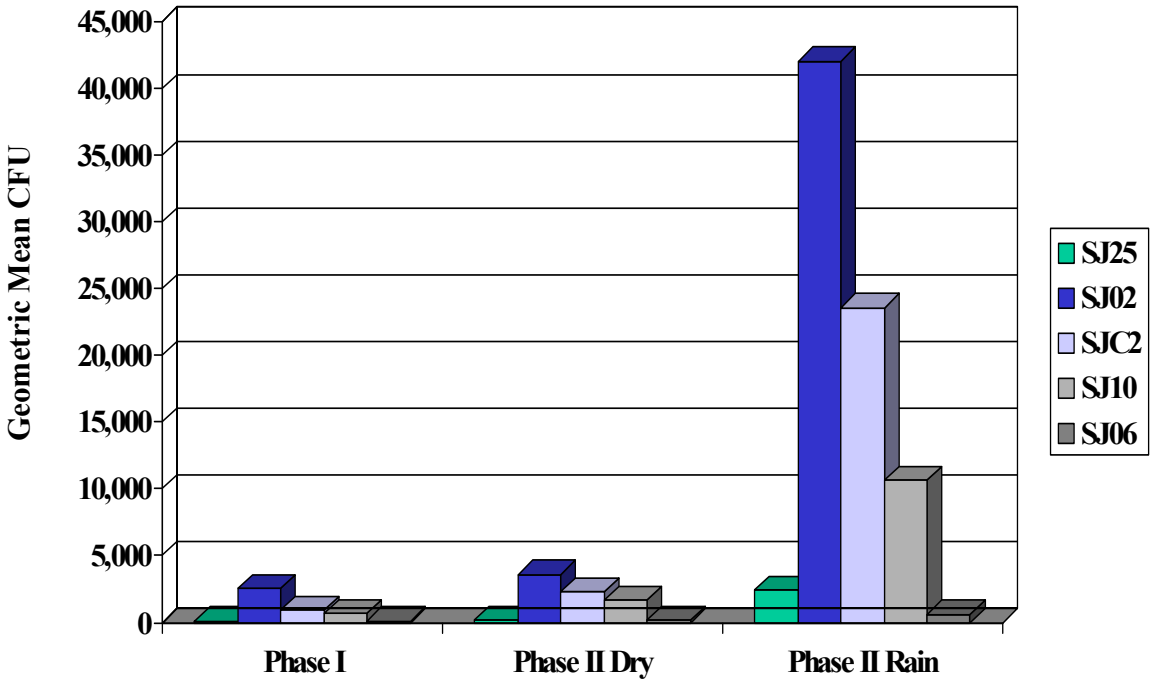

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Figure 2C –San Juan Watershed Enterococcus
Phase I (April – July) and
Phase II (September – December)

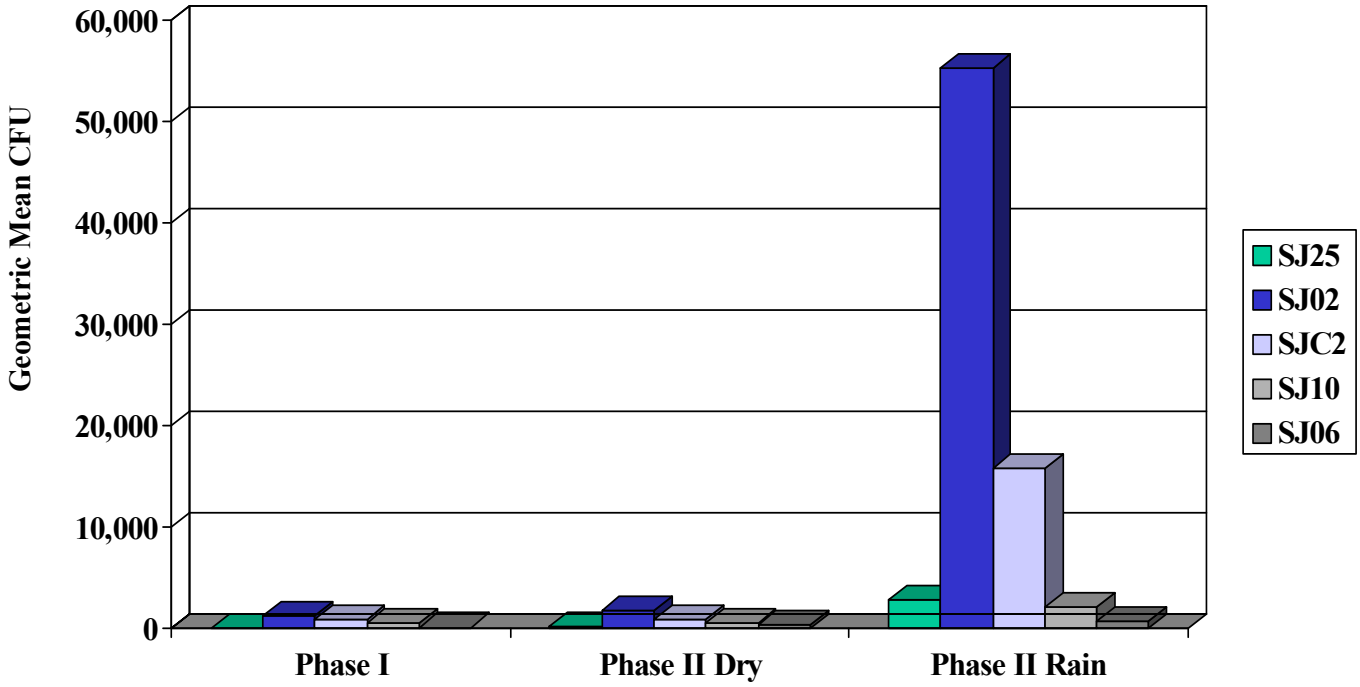

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Figure 2D –San Juan Watershed *E. coli*
Phase II (September – December)

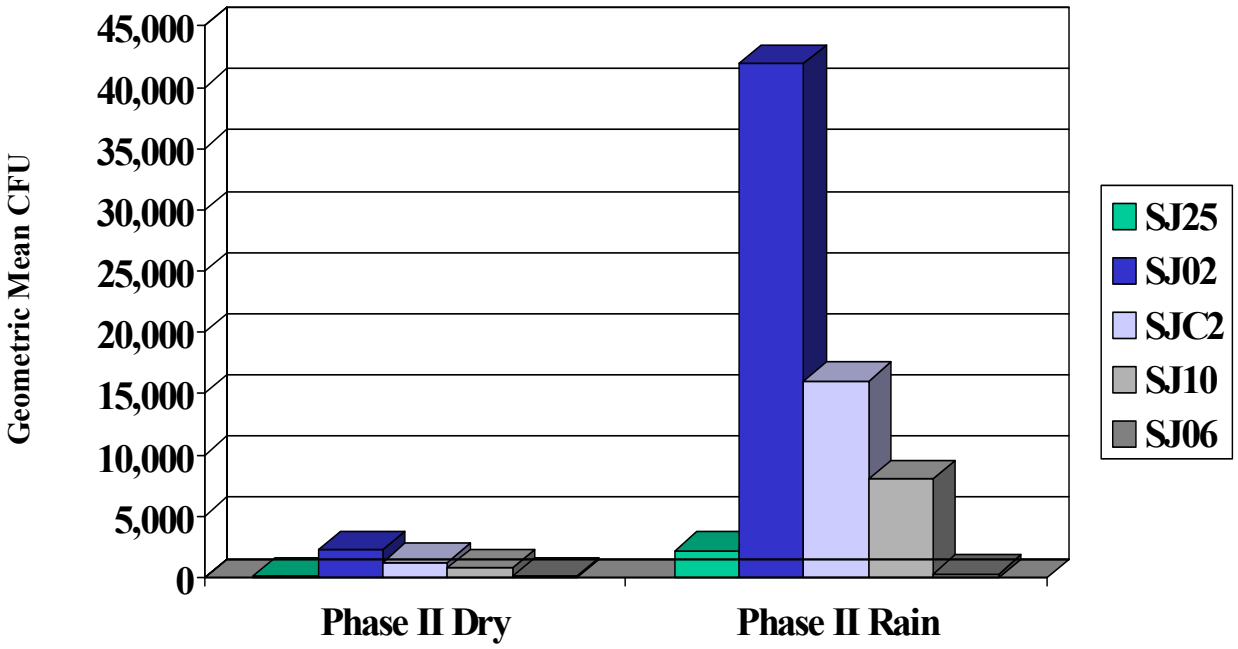

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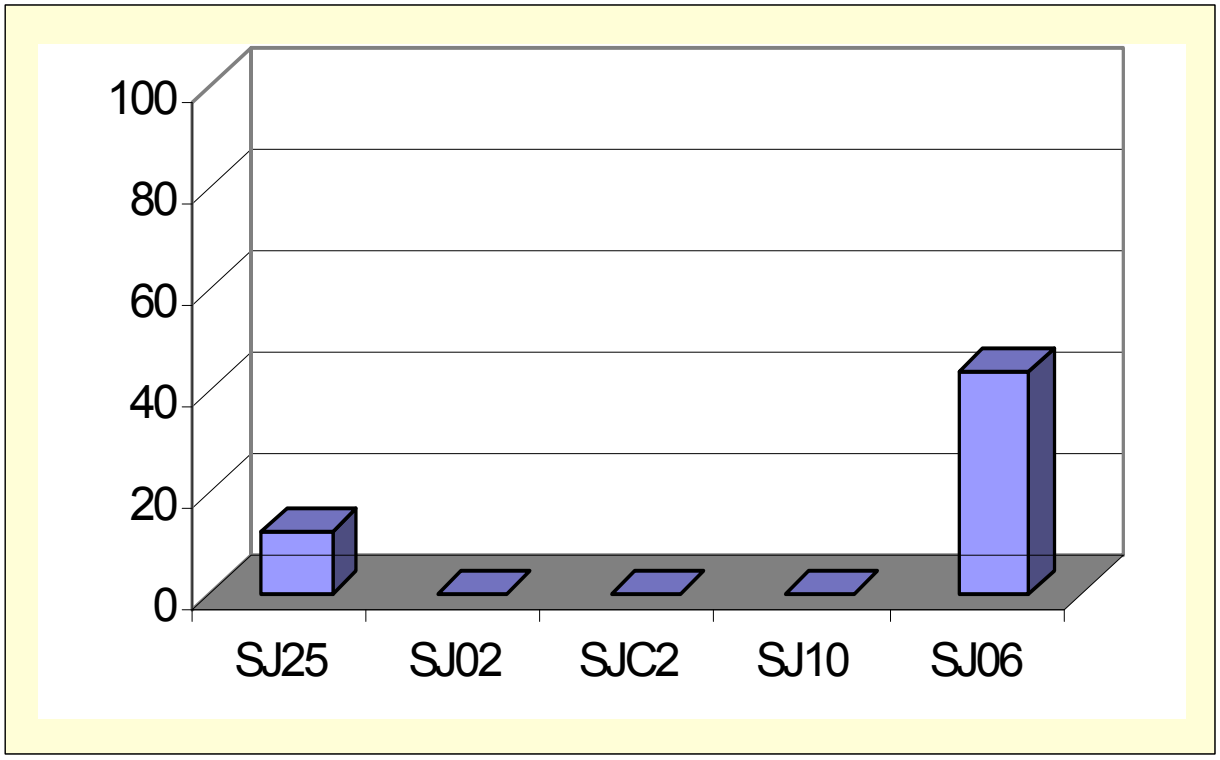
Figure 3 – San Juan Watershed Phase II: Compliance with REC-1 Standard

Based on 5-Week Average <200



% Compliant

Station

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Figure 4 – San Juan Watershed Phase II: Compliance with REC-2 Standard

Based on 5-Week Average <2000

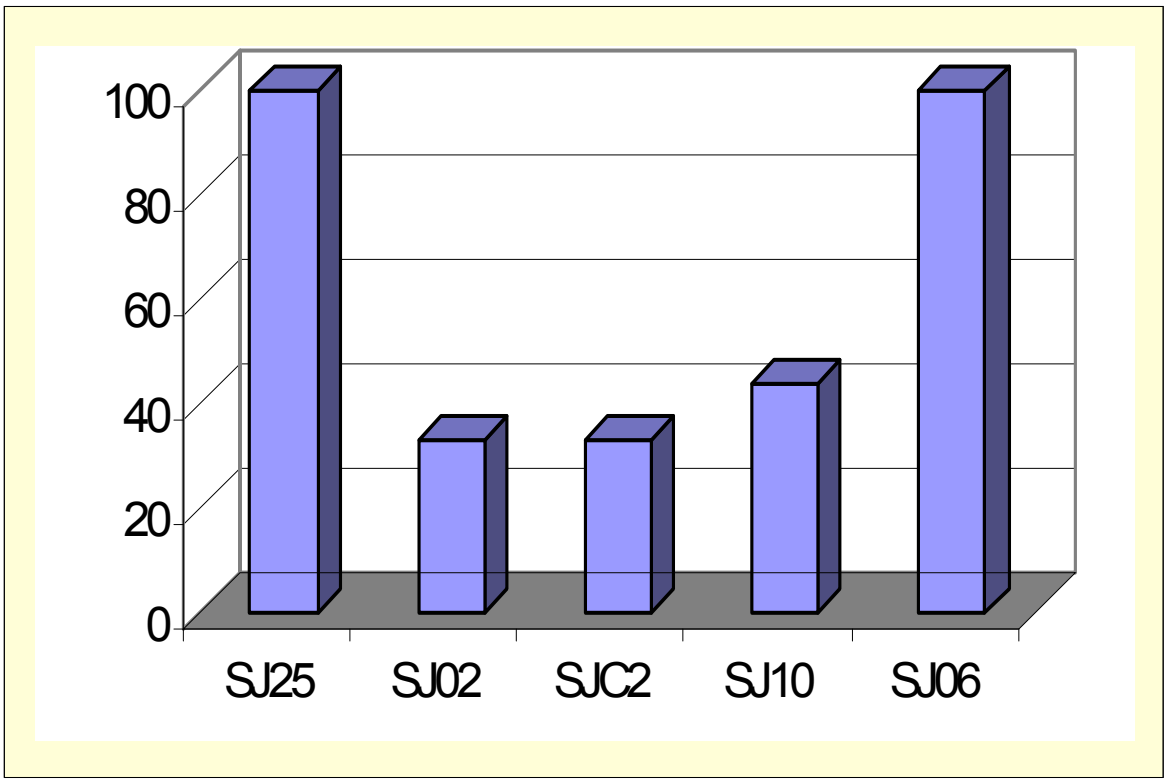

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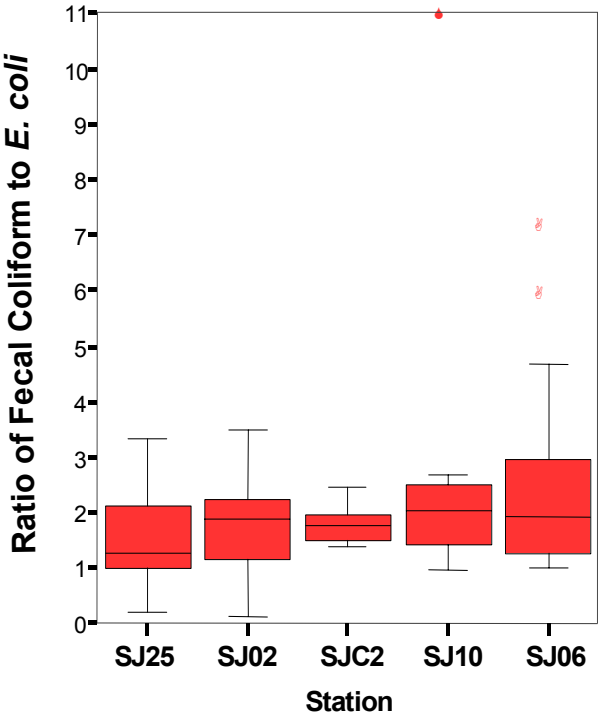
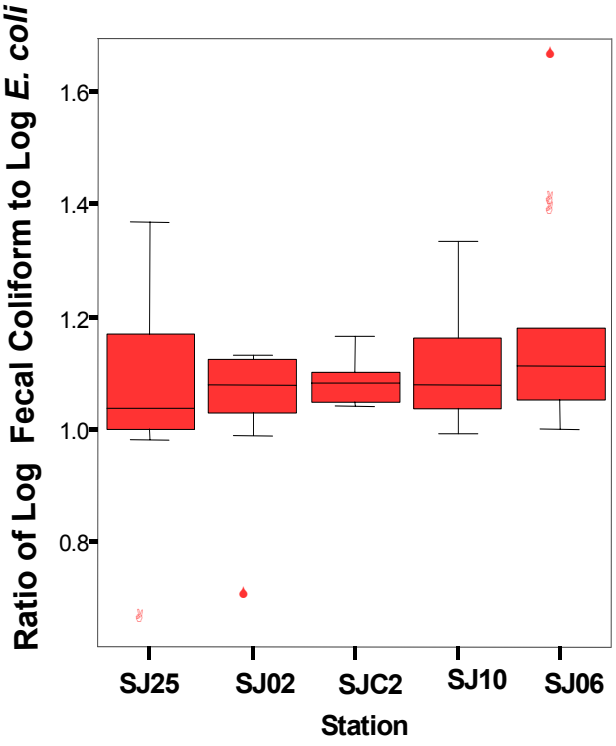
Figure 5: San Juan Watershed Phase II
Ratio of Fecal Coliform to *E. coli*





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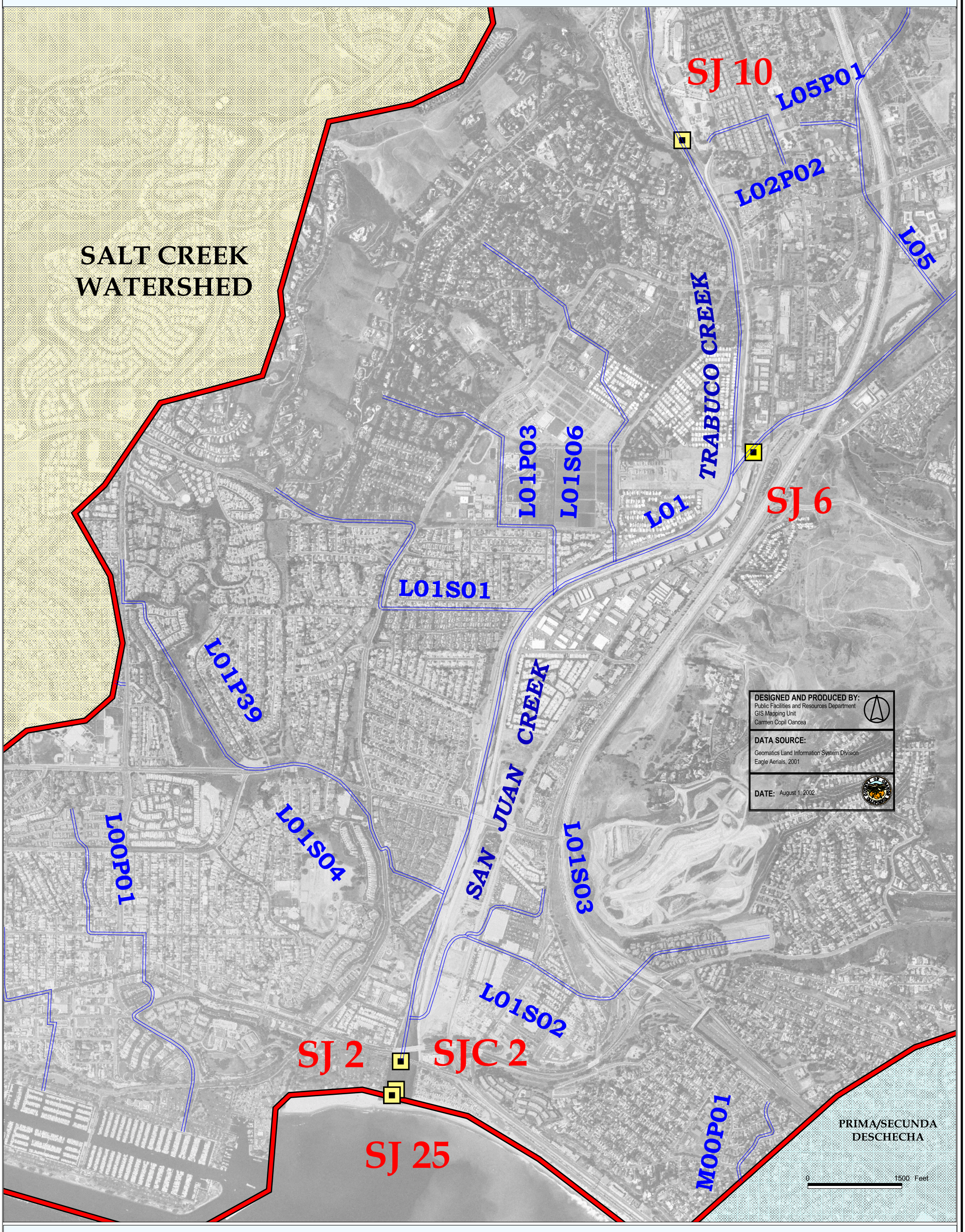
WATERSHED L : SAN JUAN CREEK



SELECTED MONITORING SITES

COUNTY OF ORANGE, CALIFORNIA

Figure 7: San Juan Creek Mouth

Figure 8: Site SJ C2 San Juan Creek at PCH

Figure 9: Site SJ 6 San Juan Creek u/s of Trabuco

Figure 10: Site SJ 10 Trabuco Creek at Ramos St. u/s of L02P02

List of tables

Table 1: San Juan Watershed Study Phase II Sampling Sites

Station number	Watershed mile	Location	Type of Sample	No. samples
SJ25	0	Ocean at San Juan Creek Mouth	Ocean	15
SJ02	0.05	San Juan Creek at Beach, East (behind berm)	Creek	13
SJC2	0.02	San Juan Creek at PCH	Creek	13
SJ06	0.24	San Juan Creek upstream of Trabuco Creek	Creek	15
SJ10	0.9	Trabuco Creek at Ramos St., u/s of L02P02	Creek	13
				Total: 69

Table 2: San Juan Creek Phase II Bacterial Concentrations

Highlighted cells represent precipitation-influenced data points, ≥ 0.20 in.
Italicized entries indicate station not bermed on sampling date

Station: SJ25 **Location: Ocean at San Juan Creek**

Indicator:

	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8	Week 9	Week 10	Week 11	Week 12	Week 13	Log Mean
Log Total Coliform	2.41	1.3	1.78	3.56	2.41	1.9	2.86	3.2	*5.7*	*2.3*	*3.83*	*3.95*	*2.54*	
Log Fecal Coliform	2	0	1.78	3.18	1.78	1.3	2.3	2.95	*4.67*	*2*	*3.2*	*3.41*	*2.08*	
Log Enterococcus	2.3	1.3	1.9	2.76	2.56	1.6	2.6	2.82	*4.58*	*2.18*	*3.41*	*3.66*	*2.18*	
Log *E. coli*	2	1.3	1.3	3.09	1.3	2	1.78	2.95	*4.76*	*2*	*2.88*	*3.26*	*1.78*	
Total Coliform	260	20	60	3600	260	80	720	1580	*500000*	*200*	*6800*	*9000*	*350*	**802.15**
Fecal Coliform	100	<20	60	1500	60	20	200	900	*47000*	*100*	*1600*	*2580*	*120*	**358.63**
Enterococcus	200	20	80	580	360	40	400	660	*38000*	*150*	*2600*	*4600*	*150*	**402.24**
E. coli	100	20	20	1220	20	100	60	900	*58000*	*100*	*760*	*1800*	*60*	**217.98**

Station: SJ02 **Location: San Juan Creek at Beach, East**

Indicator:

	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8	Week 9	Week 10	Week 11	Week 12	Week 13	Log Mean
Log Total Coliform	3.6	3.68	4.07	3.75	3.58	3.98	6.11	5.95	*4.67*	*4.48*	*4.85*	*4.46*	*4.32*	
Log Fecal Coliform	2.91	2	3.73	2.92	3.03	3.7	4.9	4.83	*4.32*	*3.81*	*4.41*	*3.58*	*4.18*	
Log Enterococcus	2.2	2.41	3.86	2.68	2.2	3.14	3.66	4.78	*4.15*	*4.03*	*4.71*	*3.53*	*3.91*	
Log *E. coli*	2.6	2.9	3.3	2.6	2.95	3.29	4.36	4.9	*4.18*	*3.53*	*4.34*	*3.23*	*3.9*	
Total Coliform	4000	4800	11800	5600	3800	9600	1300000	900000	*47000*	*30000*	*70000*	*29000*	*21000*	**26548.63**
Fecal Coliform	820	100	5400	840	1060	5000	80000	68000	*21000*	*6400*	*26000*	*3800*	*15000*	**5219.90**
Enterococcus	160	260	7200	480	160	1380	4600	60000	*14200*	*10800*	*51000*	*3380*	*8200*	**3040.66**
E. coli	400	800	2000	400	900	1960	23000	80000	*15000*	*3400*	*22000*	*1700*	*8000*	**3518.77**

Station: SJC2 **Location: San Juan Creek at PCH**

Indicator:

	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8	Week 9	Week 10	Week 11	Week 12	Week 13	Log Mean
Log Total Coliform	2.9	3.62	4.26	3.97	3.51	3.83	3.79	6.18	4.76	4.23	3.96	5.11	4.25	
Log Fecal Coliform	2.72	2.81	2.98	3.33	2.7	3.16	3.38	4.7	3.97	4.06	3.3	4.77	3.75	
Log Enterococcus	2.34	2	2.4	2.66	2.3	2.81	2.72	3.45	3.7	4.1	3.43	4.7	3.86	
Log *E. coli*	2.45	2.41	2.76	3.03	2.45	2.95	3.03	4.43	3.82	3.7	3.15	4.59	3.6	
Total Coliform	800	4200	18200	9400	3200	6800	6200	1500000	57000	17000	9200	130000	17600	**15230.40**
Fecal Coliform	520	640	960	2120	500	1460	2420	50000	9400	11600	2000	59000	5600	**3238.55**
Enterococcus	220	100	250	460	200	640	520	2820	5000	12600	2700	50000	7200	**1295.94**
E. coli	280	260	580	1080	280	900	1060	27000	6600	5000	1400	39000	4000	**1818.03**

Station: SJ10 **Location: Trabuco Creek at Ramos St. U/S of L02P02**

Indicator:

	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8	Week 9	Week 10	Week 11	Week 12	Week 13	Log Mean
Log Total Coliform	3.95	3.73	4.62	4.07	4.06	3.85	3.6	5.9	5.66	3.66	4.04	3.7	5.48	
Log Fecal Coliform	3.28	3.11	2.66	2.94	3.15	3.03	2.76	4.51	4.83	2.7	3.08	3.23	4.11	
Log Enterococcus	2.6	2.64	2.45	2.79	2.34	2.6	2.34	3.4	3.6	2.49	3.18	3.04	2.99	
Log *E. coli*	3.04	2.68	2.68	2.87	2.85	2.87	2.34	4.18	4.66	2.3	2.72	3.15	3.08	
Total Coliform	9000	5400	42000	11800	11600	7000	4000	800000	460000	4600	11000	5000	300000	**21567.79**
Fecal Coliform	1900	1300	460	880	1420	1060	580	32400	67000	500	1200	1700	13000	**2180.07**
Enterococcus	400	440	280	620	220	400	220	2510	4000	310	1500	1100	980	**639.26**
E. coli	1100	480	480	740	700	740	220	15000	46000	200	520	1400	1200	**1075.52**

Station: SJ06 **Location: San Juan Creek U/S of Trabuco**

Indicator:

	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8	Week 9	Week 10	Week 11	Week 12	Week 13	Log Mean
Log Total Coliform	4	3.87	3.98	3.99	4.03	3.97	4.1	4.9	4.9	4.11	4.23	3.75	4.7	
Log Fecal Coliform	2.3	1.3	1.3	1.3	2.15	2	2.45	3.09	3.62	2.72	2.9	2.45	3.2	
Log Enterococcus	2.49	2.51	2.36	2.45	2.38	2.3	2	2.9	3.9	2.26	3.28	2.18	3.15	
Log *E. coli*	2.15	1.3	1.3	0	1.3	1.9	1.78	2.62	3.2	1.95	2.68	2.15	2.93	
Total Coliform	10000	7400	9600	9800	10600	9400	12600	80000	79000	12800	17000	5600	50000	**15645.51**
Fecal Coliform	200	20	20	20	140	100	280	1240	4200	520	800	280	1600	**233.36**
Enterococcus	310	320	230	280	240	200	100	800	8000	180	1900	150	1400	**423.50**
E. coli	140	20	20	<20	20	80	60	420	1600	90	480	140	860	**127.69**

Table 3: San Juan Watershed Phase II: Descriptives by Sample Type

Combined Sample Types:				
	Total Coliform	**Fecal Coliform**	**Enterococcus**	***E. coli***
Number of Samples	69	68	69	68
Mean	97681	8359	4660	5650
Median	9400	1060	460	720
Minimum	20	20	20	20
Maximum	1500000	80000	60000	80000
Range	1499980	79980	59980	79980
Geometric Mean	9480	1196	816	700

Creek Samples:				
	Total Coliform	**Fecal Coliform**	**Enterococcus**	***E. coli***
Number of Samples	54	54	54	53
Mean	115078	9502	5043	6040
Median	10800	1270	500	800
Minimum	800	20	100	20
Maximum	1500000	80000	60000	80000
Range	1499200	79980	59900	79980
Geometric Mean	18424	1612	976	947

Ocean Samples:				
	Total Coliform	**Fecal Coliform**	**Enterococcus**	***E. coli***
Number of Samples	15	14	15	15
Mean	35054	3949	3281	4272
Median	720	360	400	100
Minimum	20	20	20	20
Maximum	500000	47000	38000	58000
Range	499980	46980	37980	57980
Geometric Mean	867	378	427	241

Table 4: San Juan Watershed Study Phases I and II Geometric Mean Comparative Results

	Total Coliform			Fecal Coliform			Enter ococcus			*E. coli*	
Station	Phase I	Phase II Dry	Phase II Rain	Phase I	Phase II Dry	Phase II Rain	Phase I	Phase II Dry	Phase II Rain	Phase II Dry	Phase II Rain
	April - July	Sept. - Dec.	Sept. - Dec.	April - July	Sept. - Dec.	Sept. - Dec.	April - July	Sept. - Dec.	Sept. - Dec.	Sept. - Dec.	Sept. - Dec.
SJ25	153	359	9,811	117	181	2,393	58	213	2,877	108	2,188
SJ02	9,420	17,646	250,998	2,533	3,572	42,047	1,295	1,794	55,317	2,242	41,952
SJC2	4,090	11,116	86,081	910	2,258	23,550	925	822	15,811	1,224	16,044
SJ10	4,818	18,651	47,958	695	1,633	10,672	508	515	2,098	746	8,025
SJ06	8,555	14,826	11,366	92	176	602	78	356	615	98	303

CHAPTER 3

PHASE III: FINAL SOURCE IDENTIFICATION REPORT

Contents

1. Introduction

The San Juan Creek (SJC) Watershed Bacteriological Study was conducted to characterize the fecal indicator bacteriology of the watershed and to determine the sources of bacterial pollution using a combination of bacteriologic monitoring surveys and source tracking methods. The potential sources of fecal contamination identified in the SJC watershed include humans, sewage, storm drains, waterfowl, pets, horses and wild animals. During Phase I, water samples were collected from 36 sites in the watershed and bacterial densities were determined for total and fecal coliforms and *Enterococcus*. High levels of fecal indicator bacteria were consistently found in storm drains while moderate levels were detected in all the creek samples. However, no single source of bacterial pollution was identified based on the bacteriological monitoring results. During Phase II, five sites representative of different areas of the watershed were sampled for total and fecal coliforms, *Enterococcus* and *E. coli*. Bacterial levels were used to determine temporal and geographical differences in pollution and to identify the potential sources of contamination.

In addition to conducting a monitoring study, bacterial isolates were obtained from a variety of fecal and water samples for source tracking analysis to be conducted during Phases III and IV. The objectives were to identify specific sources or host species of fecal indicator bacteria using two different types of source tracking methods, Antibiotic Resistance Analysis (ARA) and ribotyping. Dr. Valerie Harwood of the University of South Florida (USF), in Tampa, Florida conducted the ARA analysis and Dr. George Lukasik of Biological Consulting Services (BSC) in Gainesville, Florida performed the ribotyping testing. In Phase III, large numbers of bacterial isolates obtained from animal and human fecal samples were used to create *E. coli* and *Enterococcus* databases or "libraries" required for ARA and ribotyping testing. Once the libraries were constructed using isolates from known sources, the accuracy and reproducibility of the methods were evaluated using unknown isolates. The purpose of Phase IV was to determine the sources of the watershed bacterial isolates using the ARA and ribotyping methods. However, Phase IV was not completed upon determination that the accuracy for both ARA and ribotyping in identifying specific host species for *E. coli* and *Enterococcus* isolates from the SJC Watershed were not sufficient.

2. Experimental Design/Sampling Plan

A. Sample Collection and Preparation

Environmental Samples

During Phase II, Orange County Public Health Laboratory (OCPHL) staff collected and analyzed 68 water samples for total and fecal coliform, *Enterococcus*, and *E. coli*. *E. coli* and *Enterococcus* bacterial strains were isolated from the water samples collected from 5 sites (Table 1). The water samples were tested for total coliforms, fecal coliforms, *Enterococcus* and *E. coli* using the membrane filtration methods (SM9222A & B, USEPA method 1600, USEPA modified *E. coli* Method (1998), respectively) described in Standard Methods for the Examination of Water and Wastewater, 20th edition and USEPA Improved Enumeration Methods for the Recreational Water Quality Indicators: Enterococci and *Escherichia coli*. A large number of *E. coli* and Enterococci isolates (1,820 and 1,850, respectively) were frozen for source tracking analysis to be conducted during Phase IV, upon completion of ARA and ribotyping library analysis and method accuracy determination.

Library Samples

OCPHL collected human and animal fecal samples as well as sewage samples to obtain strains of *E. coli* and *Enterococcus* bacteria used to create the ARA and ribotyping libraries (Table 2). A total of 675 fecal samples (85 cat, 103 dog, 188 seagull, 109 horse, 190 human) were collected, most of which were used for both *E. coli* and *Enterococcus* testing. Sewage effluent (treated sewage) and influent (before treatment) samples were collected by South Orange County Wastewater Authority. Human fecal samples were obtained from Mission Hospital Regional Medical Center and San Clemente Hospital and Medical Center, both located within the San Juan Watershed. Cat and dog fecal samples were acquired from veterinary clinics also located near the study area. Seagull droppings were collected by OCPHL from the coastline of Doheny Beach. The fecal bacteria were isolated using CHROMagar ECC (CECC) (Hardy Diagnostics) and Enterococcosel (BBL) medias for isolation of *E. coli* and *Enterococcus*, respectively. Sewage samples were also processed using the membrane filtration method. The membranes were placed onto CHROMagar ECC (CECC) and m-EI (modified *Enterococcus*) (USPEPA Method 1600) medias for isolation of *E. coli* and *Enterococcus*, respectively. Up to five isolates each of *E. coli* and *Enterococcus* were obtained per fecal source and 10 isolates per sewage source, resulting in over 7,000 isolates. Both *E. coli* and *Enterococcus* isolates from the fecal and sewage samples were sent to the USF for ARA testing; only *E. coli* isolates were sent to BSC for ribotyping. The *E. coli* isolates tested for ribotyping were also a subset of the isolates tested by ARA.

B. Library Preparation

Both ARA and ribotyping require constructing large databases or libraries of isolate patterns based on the antibiotic resistance patterns (ARPs) or ribotypes of bacteria from known species before they can be used to identify bacteria as being human or animal-derived. Fecal sources used to create the San Juan Watershed libraries included cat, dog, horse, human, seagull, sewage influent and effluent. ARPs of bacterial isolates from these sources were determined using a battery of antibiotics at various concentrations. The ARPs were analyzed using discriminant analysis (DA) and isolates were classified according to the most likely host species source. The robustness of the library was evaluated by performing a holdout analysis. Isolates from various known sources were "held out" of the library, and were analyzed as if they were unknowns. The internal accuracy of the ARA library was measured by the average rate of correct classification (ARCC). The ARCC is the sum of the correct classifications for all source categories divided by the total number of strains in the database and expressed as a percentage.

The ribotyping library was created using ribotype (RT) profile or patterns of *E. coli* restriction fragments that were statistically analyzed for similarities and placed into "ribogroups". The percent similarity of RTs was determined using Jackknife analysis (Bionumerics software). The principle of the Jackknife method is to take out one entry or isolate from the list, and to classify it based on the maximum similarities with each group, i.e., the group with entries most similar to the entry being identified, without including the entry itself.

C. Technique Accuracy and Reproducibility Determination Using Proficiency Samples

Accuracy Testing Using Proficiency Samples

The accuracy and validity of the discriminatory function of the ARA and ribotyping methods was evaluated by comparing known *E. coli* and *Enterococcus* isolate profiles to the library profiles. *E. coli* and *Enterococcus* isolates (n=97) from known fecal and sewage samples were sent to USF as "blind" (source not identified) or proficiency samples to determine the efficiency of ARA in accurately determining the source(s) of bacterial isolates. These bacterial isolates were from samples collected concurrently with the samples used to create the libraries but kept frozen until the libraries were completed. The purpose for using isolates from known fecal sources that were not included in the library was to mimic the analysis of unknown environmental samples, while retaining the capability of judging the accuracy of the results. The same *E. coli* proficiency isolates tested to determine the accuracy of ARA were also tested by BSC using ribotyping so that the accuracy of the methods could be compared directly.

Reproducibility Testing

The purpose of the reproducibility testing was to determine whether the ARA and ribotyping methods could produce the same results in terms of classifying isolates into source categories when the testing was repeated at least 3 times using the same set of samples. Sub-sets of bacterial isolates from the proficiency samples were used to test the reproducibility of ARA and ribotyping. The same set of *E. coli* proficiency isolates was used to test the reproducibility of both methods.

The ARA reproducibility study was designed to determine the consistency of repeated measures of the antibiotic resistance patterns of a selected group of *E. coli* and *Enterococcus* isolates over time. Twenty each of *E. coli* and *Enterococcus* isolates were subjected to ARA once a week for 3 weeks. Three replicate measurements of the ARP of each isolate were obtained each week. Therefore, a total of 9 ARP measurements for each isolate were conducted (3 per week for 3 weeks).

The ribotyping reproducibility study was conducted by sub-culturing each of the 20 *E. coli* proficiency isolates in triplicate and ribotyping the samples 3 different times.

3. Data Analysis

A major component of bacterial source identification involves analyzing the different bacterial patterns using several statistical techniques. In this study, the ARA library and accuracy testing was analyzed by discriminant analysis, SAS 8.0 (SAS Institute, Cary, NC). ARA reproducibility testing and ribotyping library and accuracy determination was analyzed using the Jackknife discrimination and Pearson clustering statistical programs (Bionumeric Software, Applied Maths, Austin TX). Regardless of the type of analysis used, the efficiency of the ARA and ribotyping methods to classify known isolates into correct source groups is measured by the ARCC and rate of correct prediction (RCP). The ARCC is the sum of the correct classifications for all source categories divided by the total number of strains in the database expressed as a percentage. RCP is the percentage of isolates correctly predicted divided by the total classified for each species. Unlike other published calculations in source tracking studies, the RCP accounts for both the correct classification rate and the rate of misclassification in each source category. The higher the RCP, the more accurate the classification of isolates into a given source category.

Determination of the library accuracy for ARA differed from ribotyping in terms of the number of source groups used. Whereas the accuracy of the ribotyping library was based on the ability to correctly classify an isolate into 1 of 7 source

categories, the accuracy of the ARA library was based on using 6 categories. The 7 categories were as follows: cat, dog, horse, seagull, human, sewage influent and sewage effluent. For ARA analysis, the sewage influent and effluent results were combined into a single "sewage" category. Correct classification rates generally increase with decreasing number of source categories, as long as isolates are being grouped into valid categories.

Since *E. coli* or Enterococci isolates from sewage influent or effluent samples could potentially be classified into categories other than human, the library and proficiency results were analyzed with and without including sewage as a category. In this study, the "human" category refers to clinical isolates from human subjects.

4. Results

Various source tracking techniques have recently been used to identify sources of fecal pollution in source water, however the accuracy or robustness of these methods has not been rigorously tested in the field. In previous studies, the accuracy of ARA methods was evaluated based on how well isolates within the library or database were classified or "self-crossed" (Wiggins, et al., 1996; Harwood et al., 2000). The efficiency of the library was based on the average correct classification rates for discriminating sources. However, additional validation of the library accuracy and reproducibility was not tested using proficiency or "blind samples". Thus, in this study, the accuracy of ARA and ribotyping for identifying specific sources of *E. coli* and *Enterococcus* isolates was also evaluated using a proficiency panel comprised of 100 bacterial isolates from known source species. The internal accuracy of the library as well as the accuracy and reproducibility determination based on the proficiency panels was evaluated for both ARA and ribotyping.

A. Antibiotic Resistance Analysis

Internal Accuracy of the ARA Library

The internal accuracy of the ARA libraries for *E. coli* and *Enterococcus* libraries is shown in Table 3. The source of the fecal isolates is listed in the first column of the classification table and the assigned classifications are listed in the top row. The ARCC for *E. coli* ARA library was 43.6% based on an average correct classification of 1,517 of a total of 3,477 isolates. The RCPs ranged from 26.9% for isolates from dogs to 63.6% for sewage isolates.

The ARCC for *Enterococcus* library was 47.7% based on correctly classifying 1,746 of a total of 3,657 isolates. The RCPs ranged from 25.7% for cats to

66.7% for sewage. For both fecal indicator ARA libraries, the sewage isolates had the highest rate of correct prediction for *E. coli* and *Enterococcus*.

Accuracy of ARA Based on Proficiency Testing

Ninety-seven *E. coli* and 99 *Enterococcus* isolates from 7 fecal sources were tested as "blind" samples. The source of the isolates was unknown to the USF laboratory performing the ARA, but known to OCPHL. The analysis of correct classification is presented in Table 4. The highlighted values show the number and percentage of isolates that were identified to the assigned group. Overall, the ARCC of the *E. coli* isolates (based on testing proficiency samples) was 28.9% as compared to 43.6% for the library. The RCPs ranged from 9.1% to 100%, however, in this case, the 100% RCP result was a statistical anomaly since only 1 human *E. coli* isolate was correctly classified while 15 human isolates were misclassified. The ARCC for human *E. coli* isolates was 6.3% as compared to 39.3% for the library.

As for *Enterococcus* isolates, the ARCC of the proficiency samples was 45.5%, which reflects the library ARCC of 47.7%. Sewage and horse isolates had the highest classification rates at 85.7% and 78.6%, respectively. However, none of the 16 human isolates were classified as human; 7 isolates were misclassified as cat and 4 were classified as sewage.

Reproducibility of ARA Based on Proficiency Testing

After the accuracy testing was conducted, a subset of the 97 proficiency samples was tested to assess method reproducibility. Twenty isolates of *E. coli* and *Enterococcus* were subjected to ARA on 3 different days. Three replicates were processed per day for a total of 9 results per isolate. Table 5 lists the proficiency results (Predicted Source, Trial 1) for comparison with the reproducibility results (Predicated Source Reproducibility Trials). The reproducibility results were also analyzed without sewage (data not shown). Of the 20 *E. coli* isolates tested, 3 isolates agreed for all 9 trials, 1 of which was identified to the correct source. Ten results agreed at least 6 out of 9 times, but only 2 were correct as to source. As for *Enterococcus*, only 1 of 20 isolates was correctly identified for all 9 trials (Table 6).

B. Ribotyping

Internal Accuracy of the Ribotyping Library

The *E. coli* ribotyping library was constructed based on the ribotype profile of 997 isolates that were also included in the ARA database. The proficiency of the library is presented in Table 7 as the "Maximum Similarity Jackknife Analysis of *E. coli* Ribotype Profiles". The source of the fecal isolates is listed in the first column of the classification table and the assigned classifications or categories

are listed in the top row. The range of percentage of maximum similarity ranged from 33.6% for effluent to 82.4% for horses. The ARCC for human isolates was 75.5%. Overall, the ARCC for *E. coli* using 7 sources was 63.8%.

Accuracy of Ribotyping Based on Proficiency Testing

The same set of 97 "blind" *E. coli* isolates analyzed for ARA accuracy testing was also used to determine the accuracy of the ribotyping method. Overall, the ARCC was 26.8% (ranging from 7.1% for sewage to 62.5% for human isolates) (Table 8). The ARCC did not change significantly when the results were also analyzed without including the sewage category (29.0% ARCC, data not shown). Based on testing the *E. coli* proficiency isolates, the accuracy levels of ribotyping (26.8%) and ARA (28.9%) were very similar overall. However, the level of accuracy for classifying human *E. coli* isolates was significantly better using ribotyping (62.5% ARCC) as compared to ARA (6.3%).

Reproducibility of Ribotyping Based on Proficiency Testing

Twenty "blind" proficiency isolates were tested in triplicate and identified to 1 of 7 possible source categories (Table 9). Of the 20 isolates tested, 2 isolates (10%) were correctly classified for all 3 reproducibility trials. Thirteen of 20 isolates (65%) were identified as the same source all 3 times, 2 of which was identified as the correct source. Five isolates agreed for 2 of 3 trials (66% > 100%), 2 of which were correctly classified. There was no significant difference in the results when the data was analyzed without including sewage isolates (data not shown).

C. Comparison of ARA and Ribotyping Results

Agreement Between ARA and Ribotyping

The agreement between the ARA and ribotyping methods was compared using the *E. coli* proficiency results (N=97). For both methods, only 6 of 97 isolates (6%) had identical and correct classifications: horse (N=3), human (N=1), cat (N=1) and dog (N=1). There was no significant difference in agreement between ARA and ribotyping when sewage was excluded as a category.

Reproducibility

The reproducibility testing of 20 isolates by ARA and ribotyping are summarized in Table 10. Ribotyping was superior to ARA in terms of reproducing the source of isolates. However due to the low accuracy level, in most trials the predicted source was not correctly identified.

Classification as Human and Sewage vs. Non-human Sources

The ability of ARA and ribotyping for classification *E. coli* and *Enterococcus* isolates as human and sewage vs. non-human group was compared. An ideal identification method is accurate, highly sensitive and specific. In this comparison human and sewage isolates were combined into one group while the cat, dog, horse and seagull isolates were pooled as the non-human group. The accuracy, sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) for both methods are presented in Table 11. Sensitivity is the fraction of human and sewage isolates that were correctly classified while specificity is the fraction of non-human isolates correctly identified. The PPV represents the percentage of human and sewage isolates identified as such. The NPV represents the percentage of non-human isolates identified as such. Accuracy is the sum all of correct classifications divided by the total number of isolates tested. The overall accuracy for classifying isolates as human and sewage vs. animal derived was 57% for *E. coli* and 60% for *Enterococcus* using ARA and 67% for *E. coli* using ribotyping. The ribotyping method had higher sensitivity, specificity, PPV, NPV and accuracy, as compared to ARA for correctly classifying *E. coli* isolates as human and sewage or non-human in origin. However, this result can be attributed to the fact that more human isolates were correctly classified by ribotyping (62.5%) as compared sewage isolates (7.1%). Similarly, the ARA method had the highest sensitivity (66%) for classifying *Enterococcus* as human and sewage, but this result reflects how well sewage isolates were classified (85.7%) as compared to 0% of the human isolates (Table 4). Ribotyping may be superior to ARA for discriminating *E. coli* isolates from humans as being “human and sewage”. On the other hand, ARA may be more useful for classifying *Enterococcus* from sewage into this combined category.

5. Source Identification of Watershed Isolates

The watershed isolates collected during Phase II were not tested using ARA and ribotyping due to the low accuracy and reproducibility results obtained using proficiency isolates. The ARCC for *E. coli* was 29% for ARA and 27% for ribotyping as determined using 97 proficiency isolates. The probability of correctly classifying a given isolate by chance for ribotyping was one in seven (categories), or 14.3%. While the 27% correct classification rate for proficiency isolates by ribotyping is nearly twice the expected rate by chance, this is significantly lower than 75% or higher rate that was anticipated at the beginning of the study. In the case of ARA, the probability of correctly classifying a given isolate by chance was one in six (categories), or 16.7%. While the 29% correct classification rate of proficiency isolates by ARA also represents nearly twice the rate expected by chance, the accuracy is far below acceptable limits. The accuracy of ARA using *Enterococcus* was also low (ARCC, 46%). Both methods

showed poor reproducibility testing 20 isolates. Therefore, it was determined that for this study, the ARA and ribotyping methods would not be useful for accurately classifying *E. coli* and *Enterococcus* isolates as originating from dogs, cats, horses, seagulls, human or sewage effluent and influent.

6. Discussion

The basis for bacterial source tracking procedures is the assumption that there are species-specific strains of bacteria inhabiting the intestinal tract of humans and animals. Such species-specificity would need to extend over a wide geographic area to be useful for identifying sources of bacterial contamination. Given this assumption, for ARA, specificity would be based on differential exposure to antimicrobial agents, whereas for ribotyping or other DNA typing methods, it would be based on unknown host-specific factors. Currently, the major limitation to using these techniques is the lack of research supporting species-specificity. The validity of terms such as “resident” or “transient” bacterial strains used in previous publications is still questionable. It has not yet been well established that there are resident strains shared by a large percentage of an animal or human species or that there are fecal bacteria species specific to one species’ intestinal tract.

Techniques commonly used to type isolates in epidemiological investigations of outbreaks such as pulsed-field gel electrophoresis (PFGE), ribotyping, serotyping and antimicrobial susceptibility testing are accepted by the scientific community due to extensive documentation in the literature. However, the efficiency of these techniques for identifying sources of fecal indicator bacteria in watersheds cannot be compared directly with epidemiological outbreak investigations. Whereas typing methods are used in epidemiological studies to identify unknown strains of organisms with a known source strain, in watershed source tracking studies they are used to identify patterns specific to bacteria in individual species. Further studies are also needed to determine bacterial population variation by host and geographic location to establish the potential use of source tracking techniques for watershed studies.

There are still a number of variables associated with source tracking methods that have not been established. These include the number of sources which must be discerned, the size of the known source database (library), organism used, number and types of host categories, number of isolates per known sources tested, type of statistical analysis used for data interpretation and variability due to geographic location. The libraries constructed in this study, particularly for ribotyping, were much larger and more specific as compared to libraries in previous studies (Hartel et al., 1999; Carson et al., 2001; Wiggins, 1996). Theoretically, large libraries consisting of thousands of isolates should result in high classification rates, as they are more representative of microbial populations

than small libraries. Small libraries of less than 300 isolates per source will result in high correct classification rates which can be shown to be an artifact by assessing the extent of random clustering and does not occur with large libraries (Whitlock et al., 2002). However, the optimal database size to achieve maximum accuracy of classification has yet to be determined.

The lack of standardized methods for database construction, analysis and interpretation of the results complicates the comparison of classification rates obtained from various studies. For example, in this study, 44% of *E. coli* 3477 library isolates and 48% of 3657 *Enterococcus* isolates were correctly classified into 6 source groups using ARA and discriminant analysis. These results are much lower than the 84% and 87% ARCCs reported by Wiggins (1999) and Hagedorn (1999) using the same method but with fecal streptococci as the bacterial indicator. However, a low ARCC (34%) was also reported in a similar study analyzing 319 *E. coli* isolates from 9 source categories (Guan et al., 2002). The differences in classification rates between various studies may be attributed to the types of antibiotics used, and changes in antibiotic resistance patterns of bacteria as a result of antibiotic treatment, dietary changes of the host as well as geographic differences. Guan attributed the 35% ARCC (as compared to the results obtained by Wiggins and Hagedorn) to three major factors: using *E. coli* rather than fecal streptococci or *Enterococcus* spp., types of antibiotics used and differences in diversities of the bacterial collections due to different sampling protocols, and sampling from a wider geographic area.

As for the ribotyping library results, 64% of 997 *E. coli* library isolates were correctly classified into 1 of 6 source categories, comparable to the 74% ARCC reported by Carson (2001) using 287 *E. coli* isolates and 8 source categories.

In this study, the ARCCs of ARA and ribotyping for proficiency isolates were much lower than the ARCCs of the respective libraries. Although the ARCC of the *E. coli* ARA library was 44%, only 29% of the proficiency isolates were correctly classified. Similarly, the ARCC of the *E. coli* proficiency ribotyping library was 64%, but only 27% of the proficiency isolates were correctly classified. In contrast, for *Enterococcus* the library and proficiency results were very similar. The ARCCs of the *Enterococcus* ARA library was 48% and 46% for proficiency isolates. One possible explanation for the differences in ARCC between the two indicators may be due to higher strain variability of *E. coli* versus *Enterococcus*, as well as a broader distribution of *E. coli* strains between different host groups

Accurate source determination can be difficult if the bacterial strains analyzed are very similar genetically. Closely related strains from different host species may be classified into the same category. On the other hand, identical strains with minute genetic differences may be misclassified into different categories (Parveen et al., 1999). Strain variation can also add difficulty to achieving highly reproducible results. The reasons for the low reproducibility results obtained in

this study using both methods have not yet been determined, but may be due in part to incomplete precision of the methodology.

To maximize the accuracy and representativeness of the libraries, *E. coli* and *Enterococcus* were isolated from human, animal and sewage samples within the vicinity of the San Juan Watershed. In this study, sewage influent and effluent were analyzed as source categories because public health officials are interested in the use of source tracking methods to determine sewage contamination. Interestingly, many of the *E. coli* and *Enterococcus* isolates from sewage were not classified as human-derived. However, because human isolates were obtained from hospital specimens, they may be different from human isolates from the community. Thus, the source of isolates used to construct the database may also affect classification rates.

Classification rates will also vary depending on the number of source categories used. Previous studies showed that ARCCs improved when source categories were combined (Wiggins et al., 1999; Guan et al., 2002). In this study, combining groups, such as dog and cat into a "pet" category increased the ARCC using ARA for the combined source category as compared to the individual category (data not shown). However, the disadvantage to pooling categories is the inability to track indicators to a specific animal group, although in some cases, discrimination to 3 categories (human, livestock and wildlife) may be sufficient for making management decisions. Therefore, the usefulness of ARA and ribotyping will also depend on the degree of species level discrimination necessary to provide sufficient information to watershed managers.

Bacterial typing methods may be more successful for tracking fecal sources in small, simple watersheds or geographic areas impacted by a few species (i.e., cow, wildlife and human) and with limited genetic variability. The San Juan Creek watershed is a large, complex watershed that encompasses highly urbanized and industrial areas, horse stables and rural regions. The lower end of SJC is a habitat for a variety of birds that can number in the hundreds. It is possible that the diversity of bacterial strains in this watershed is higher compared to those in other source tracking studies. The results of this study suggest that source tracking methods may not work as well for large watersheds impacted by numerous fecal sources as compared to confined areas impacted by fewer sources.

Most published source tracking studies were conducted using a single typing technique. In this study, two different typing methods were compared in terms of source classification using the same set of *E. coli* isolates. ARA classifies indicator organisms into pre-determined groups (host source categories) according to differences in antibiotic resistance patterns, whereas ribotyping is based on differences in genetic patterns. The results indicate that the methods were not comparable for classifying *E. coli* into the source categories selected for this study. Of the 97 *E. coli* isolates that were tested, only 6 were classified to the

same sources by both methods. The ARA and ribotyping methods also differed in their ability to classify human and sewage isolates. The ribotyping method was significantly better than ARA for classifying human *E. coli* isolates correctly as compared to ARA. However, ARA was superior for classifying *Enterococcus* and *E. coli* isolates from sewage. Thus, further investigation to assess the usefulness of ARA combined with ribotyping to improve source identification is needed.

7. Conclusions

1. In this study, the ARA and ribotyping methods did not demonstrate sufficient accuracy, discriminatory power, or reproducibility necessary to identify *E. coli* and *Enterococcus* isolates as originating from humans or animals, or to further discriminate isolates from specific groups such as dogs, cats, horses, seagulls, sewage and humans.

2. The accuracy levels of ARA and ribotyping should not be based solely on the internal accuracy of the library. Validation of source tracking methods should include accuracy testing using unknown isolates that are not part of the original database and are provided by an independent laboratory.

3. Source tracking methods are developing technologies that have not been rigorously tested. The theoretical basis for the techniques has not been well established. Additional investigation is needed to address critical factors such as the monitoring design, type of indicator bacteria used, size and representativeness of the database, number of fecal indicator sources, number of proficiency test samples, type of data analysis used to interpret source identification results, bacterial variation, and geographic differences.

4. ARA and ribotyping may be more successful in source tracking investigations of confined areas, with few potential sources of bacterial pollution (as demonstrated in previous studies). Further research is needed to assess the accuracy of these techniques before they are used on a routine basis to determine specific sources of pollution or remediation measures.

5. The accuracy of Bacterial Source Tracking (BST) methods as source assessment tools has not been well established, particularly in California watersheds. Watershed source identification studies should continue using intense environmental monitoring of fecal indicators to determine sources of pollution. To date, source tracking results obtained from BST methods should be interpreted cautiously to avoid implementing pollution prevention which may not be cost-effective or successful in watershed remediation efforts.

Table 1. *E. coli* and *Enterococcus* Isolates from Environmental Water Samples Collected at San Juan Creek (SJC).

Station number	Sample Site	*Escherichia coli*		*Enterococcus spp.*	
		No. samples	No. isolates	No. samples	No. isolates
SJ 02	Pacific Ocean at mouth of SJC	12	397	12	340
SJ C2	East side of SJC at the mouth	12	423	12	367
SJ 06	SJC below Pacific Coast Hwy	15	249	15	381
SJ 10	SJC above Trabuco Creek	13	406	13	375
SJ 25	Trabuco Creek	16	345	16	387
	Total	68	1820	68	1850

Table 2. Sources of *E. coli* and *Enterococcus* Isolates for Assemblage of ARA and Ribotyping Libraries.

	Escherichia coli			*Enterococcus spp.*	
Source	**No. Fecal Samples**	**No. Isolates for ARA Library**	**No. Isolates for Ribotyping Library**	**No. Fecal Samples**	**No. Isolates for ARA Library**
Human	109	523	159	160	773
Cat	64	380	110	38	299
Dog	77	423	135	78	434
Seagull	157	693	157	148	682
Horse	92	497	159	81	400
Sewage (Influent)	53	480	155	54	553
Sewage (Effluent)	52	474	155	49	516
Totals	604	3470	1030	608	3657

Table 3. Internal Accuracy of ARA Library.

Classification of *Escherichia coli* and Enterococci known isolates by source.

Number (%) of Isolates Classified As:							
E. coli							
Source ↓	**Cat**	**Dog**	**Horse**	**Seagull**	**Human**	**Sewage**	**Total**
Cat	**151 (39.7%)**	104 (27.4%)	44 (11.6%)	16 (4.2%)	39 (10.3%)	26 (6.8%)	380
Dog	71 (16.8%)	**185 (43.7%)**	39 (9.2%)	46 (10.9%)	57 (13.5%)	25 (5.9%)	423
Horse	7 (1.4%)	65 (13.1%)	**285 (57.3%)**	48 (9.7%)	5 (1.0%)	87 (17.5%)	497
Seagull	39 (5.6%)	143 (20.6%)	101 (14.6%)	**276 (39.8%)**	83 (12.0%)	51 (7.4%)	693
Human	57 (10.7%)	99 (18.6%)	42 (7.9%)	79 (14.8%)	**209 (39.3%)**	46 (8.6%)	532
Sewage	36 (3.8%)	91 (9.6%)	234 (24.6%)	99 (10.4%)	81 (8.5%)	**411 (43.2%)**	952
Total	361	687	745	564	474	646	**3477**
RCP[a]	**41.8%**	**26.9%**	**38.3%**	**48.9%**	**44.1%**	**63.6%**	
						ARCC[b]	**43.6%**
Enterococci							
Source ↓	**Cat**	**Dog**	**Horse**	**Seagull**	**Human**	**Sewage**	**Total**
Cat	**104 (34.8%)**	78 (26.1%)	13 (4.3%)	43 (14.4%)	38 (12.7%)	23 (7.7%)	299
Dog	75 (17.3%)	**168 (38.7%)**	16 (3.7%)	90 (20.7%)	38 (8.8%)	47 (10.8%)	434
Horse	9 (2.3%)	8 (2.0%)	**302 (75.5%)**	23 (5.8%)	14 (3.5%)	44 (11.0%)	400
Seagull	61 (8.9%)	75 (11.0%)	32 (4.7%)	**326 (47.8%)**	105 (15.4%)	83 (12.2%)	682
Human	98 (12.7%)	88 (11.4%)	38 (4.9%)	187 (24.2%)	**272 (35.2%)**	90 (11.6%)	773
Sewage	58 (5.4%)	60 (5.6%)	181 (16.9%)	135 (12.6%)	61 (5.7%)	**574 (53.7%)**	1069
Total	405	477	582	804	528	861	**3657**
RCP	**25.7%**	**35.2%**	**51.9%**	**40.5%**	**51.5%**	**66.7%**	
						ARCC	**47.7%**

[a]Rate of Correct Prediction

[b]Average Rate of Correct Classification

Table 4. ARA Accuracy.

Classification of *Escherichia coli* and Enterococci proficiency isolates by source.

Number (%) of Isolates Classified As:							
E. coli							
Source ↓	**Cat**	**Dog**	**Horse**	**Seagull**	**Human**	**Sewage**	**Total**
Cat	**4 (28.6%)**	3 (21.4%)	4 (28.6%)	2 (14.3%)	0 (0.0%)	1 (7.1)	14
Dog	1 (7.1%)	**1 (7.1%)**	3 (21.4%)	2 (14.3%)	0 (0.0%)	7 (50.0%)	14
Horse	0 (0.0%)	1 (9.1%)	**8 (72.7%)**	1 (9.1%)	0 (0.0%)	1 (9.1%)	11
Seagull	0 (0.0%)	2 (14.3%)	4 (28.6%)	**5 (35.7%)**	0 (0.0%)	3 (21.4%)	14
Human	1 (6.3%)	2 (12.5%)	5 (31.3%)	3 (18.8%)	**1 (6.3%)**	4 (25.0%)	16
Sewage	2 (7.1%)	2 (7.1%)	11 (39.3%)	4 (14.3%)	0 (0.0%)	**9 (32.1%)**	28
Total	8	11	35	17	1	25	**97**
RCP[a]	50.0%	9.1%	22.9%	29.4%	100%	36.0%	
						ARCC[b]	**28.9%**
Enterococci							
Source ↓	**Cat**	**Dog**	**Horse**	**Seagull**	**Human**	**Sewage**	**Total**
Cat	**3 (23.1%)**	3 (23.1%)	1 (7.7%)	0 (0.0%)	2 (15.4%)	4 (30.8%)	13
Dog	1 (7.1%)	**5 (35.7%)**	0 (0.0%)	1 (7.1%)	3 (21.4%)	4 (28.6%)	14
Horse	1 (7.1%)	0 (0.0%)	**11 (78.6%)**	0 (0.0%)	1 (7.1%)	1 (7.1%)	14
Seagull	0 (0.0%)	1 (7.1%)	1 (7.1%)	**2 (14.3%)**	2 (14.3%)	8 (57.1%)	14
Human	7 (43.8%)	1 (6.3%)	3 (18.8%)	1 (6.3%)	**0 (0.0%)**	4 (25.0%)	16
Sewage	0 (0.0%)	0 (0.0%)	1 (3.6%)	2 (7.1%)	1 (3.6%)	**24 (85.7%)**	28
Total	12	10	17	6	9	45	**99**
RCP	**25.0%**	**50.0%**	**64.7%**	**33.3%**	**0%**	**53.3%**	
						ARCC	**45.5%**

[a]Rate of Correct Prediction

[b]Average Rate of Correct Classification

Table 5. ARA *E. coli* Reproducibility.

Classification of *E. coli* proficiency isolates by source. 6-Category Analysis[a].

Isolate No.	True Source	Predicted Source Trial #1	Predicted Source Reproducibility Trials[b] (n=9)
1	CAT	SEAGULL	**3C**, 3D, 2G, 1HO
2	CAT	HORSE	**1C**, 4D, 4G
3	CAT	DOG	**1C**, 6D, 2 G
4	DOG	SEWAGE	**9D**
5	DOG	SEWAGE	1C, **4D**, 3G, 1HO
6	DOG	HORSE	2C, 3HO, 4S
7	EFFLUENT	**SEWAGE**	7D, 2 HO
8	EFFLUENT	DOG	9D
9	HORSE	**HORSE**	2G, **7HO**
10	HORSE	**HORSE**	9HO
11	HORSE	**HORSE**	1D, 4G, **4HO**
12	HUMAN	CAT	8C, **1HU**
13	HUMAN	SEWAGE	8G, 1S
14	HUMAN	SEWAGE	6D, 3G
15	INFLUENT	SEAGULL	6C, 2G, 1HO
16	INFLUENT	HORSE	2G, 4HO, **3S**
17	INFLUENT	**SEWAGE**	7D, 2HO
18	SEAGULL	HORSE	**1G**, 8HO
19	SEAGULL	**SEAGULL**	2C, 2D, **3G**, 2HO
20	SEAGULL	**SEAGULL**	2D, **7 G**

[a]Effluent and Influent combined as “Sewage” category
[b]C=cat, D=dog, G=gull, HO=horse, HU=human, S=sewage.

Table 6. ARA *Enterococcus* Reproducibility.

Classification *of Enterococcus* proficiency isolates by source. Six-category analysis[a].

Isolate No.	True Source	Predicted Source Trial #1	Predicted Source Reproducibility Trials[b] (n=9)
1	CAT	SEWAGE	5C, 4S
2	DOG	DOG	**3D, 5HO, 1S**
3	DOG	SEAGULL	4G, 5S
4	DOG	HUMAN	4HU, 5S
5	EFFLUENT	**SEWAGE**	4D, 5S
6	EFFLUENT	SEWAGE	5G, 4S
7	EFFLUENT	**SEWAGE**	9S
8	HORSE	**HUMAN**	2D, 7S
9	HORSE	**SEWAGE**	1D, 2HO, 2HU, 1G, 3S
10	HORSE	**CAT**	1C, 6D, 1S
11	HUMAN	SEWAGE	9S
12	HUMAN	CAT	6C, 3D
13	HUMAN	SEWAGE	1C, 8HO
14	INFLUENT	SEWAGE	1D, 5HU, 3S
15	INFLUENT	SEWAGE	3G, 6S
16	INFLUENT	SEWAGE	6G, 3S
17	SEAGULL	HORSE	9HO
18	SEAGULL	SEWAGE	8C, 1D
19	SEAGULL	HUMAN	2HU, 7S

[a]Effluent and Influent combined as “Sewage” category
[b]C=cat, D=dog, G=gull, HO=horse, HU=human, S=sewage.

Table 7. Internal Accuracy of Ribotyping Library.

Classification of known *Escherichia coli* isolates by source.

Number (%) Maximum Similarity Jackknife Analysis of *E. coli* Ribotype Profiles								
Source ↓	**Cat**	**Dog**	**Horse**	**Seagull**	**Human**	**Influent**	**Effluent**	**Total**
Cat	**80 (68.8%)**	16 (20.0%)	1 (1.1%)	3 (2.2%)	11 (9.7%)	5 (4.3%)	0 (0.0%)	116
Dog	17 (13.8%)	**83 (67.0%)**	2 (1.8%)	8 (6.4%)	5 (3.7%)	7 (5.5%)	2 (1.8%)	124
Horse	2 (1.3%)	2 (1.3%)	**131 (82.4%)**	4 (2.5%)	0 (0.0%)	9 (5.7%)	11 (6.9%)	159
Seagull	4 (2.6%)	10 (6.4%)	7 (4.5%)	**108 (68.8%)**	13 (8.3%)	5 (3.2%)	10 (6.4%)	157
Human	12 (7.6%)	8 (5.0%)	1 (0.6%)	11 (6.9%)	**120 (75.5%)**	4 (2.5%)	3 (1.9%)	159
Influent	3 (2.2%)	13 (8.8%)	21 (14.7%)	12 (8.1%)	8 (5.2%)	**69 (46.3%)**	22 (14.7%)	148
Effluent	5 (4.0%)	16 (12.0%)	17 (12.8%)	12 (8.8%)	12 (8.8%)	27 (20.0%)	**45 (33.6%)**	134
Total	123	148	180	158	169	126	93	**997**
RCP[a]	**65.0%**	**56.1%**	**72.8%**	**68.4%**	**71.0%**	**54.8%**	**48.4%**	
							ARCC[b]	**63.8%**

[a]Rate of Correct Prediction

[b]Average Rate of Correct Classification

Table 8. Ribotyping Accuracy.

Classification of *Escherichia coli* proficiency isolates by source.

Number (%) of *E. coli* Isolates Assigned As:							
Source↓	**Cat**	**Dog**	**Horse**	**Seagull**	**Human**	**Sewage**	**Total**
Cat	**2 (14.3%)**	5 (35.7%)	3 (21.4%)	3(21.4%)	0 (0.0%)	1 (7.1%)	14
Dog	3 (21.4%)	**5 (35.7%)**	0 (0.0%)	4 (28.6%)	0 (0.0%)	2 (14.3%)	14
Horse	2 (18.2%)	2 (18.2%)	**4 (36.4%)**	0 (0.0%)	0 (0.0%)	3 (27.3%)	11
Seagull	2 (14.3%)	4 (28.6%)	1 (7.1%)	**3 (21.4%)**	3 (21.4%)	1 (7.1%)	14
Human	1 (6.3%)	3 (18.8%)	0 (0.0%)	0 (0.0%)	**10 (62.5%)**	2 (12.5%)	16
Sewage Influent	0 (0.0%)	2 (14.3%)	0 (0.0%)	4 (28.6%)	7 (50.0%)	**1 (7.1%)**	14
Sewage Effluent	2 (14.3%)	4 (28.6%)	2 (14.3%)	4 (28.6%)	1 (7.1%)	**1 (7.1%)**	14
Total	12	25	10	18	21	11	**97**
RCP[a]	**16.7%**	**20.0%**	**40.0%**	**16.7%**	**47.6%**	**18.2%**	
						ARCC[b]	**26.8%**

[a]Rate of Correct Prediction

[b]Average Rate of Correct Classification

Table 9. Ribotyping *E. coli* Reproducibility [a].

Classification *of E. coli* proficiency isolates by source.

Isolate No.	True Source	Predicted Source Trial #1	Predicted Source Trial #2	Predicted Source Trial #3
1	CAT	SEAGULL	CAT	CAT
2	CAT	DOG	DOG	EFFLUENT
3	CAT	HORSE	CAT	CAT
4	DOG	DOG	DOG	DOG
5	DOG	INFLUENT	INFLUENT	INFLUENT
6	DOG	EFFLUENT	EFFLUENT	EFFLUENT
7	EFFLUENT	HORSE	HORSE	HORSE
8	EFFLUENT	INFLUENT	INFLUENT	INFLUENT
8	HORSE	DOG	EFFLUENT	EFFLUENT
10	HORSE	EFFLUENT	EFFLUENT	EFFLUENT
11	HORSE	EFFLUENT	DOG	SEAGULL
12	HUMAN	HUMAN	CAT	CAT
13	HUMAN	INFLUENT	CAT	SEAGULL
14	HUMAN	INFLUENT	INFLUENT	INFLUENT
15	INFLUENT	SEAGULL	SEAGULL	SEAGULL
16	**INFLUENT**	**INFLUENT**	**INFLUENT**	**INFLUENT**
17	INFLUENT	HUMAN	HUMAN	HUMAN
18	SEAGULL	DOG	DOG	DOG
19	SEAGULL	CAT	CAT	CAT
20	SEAGULL	INFLUENT	INFLUENT	INFLUENT

[a]**Based Pearson's Coefficient Correlation (2.0% tolerance.** Influent and effluent isolates were not used in the library data set for unknown identification).

Table 10. Summary of ARA and Ribotyping Reproducibility.

Method **Organism** **(No. trials)**	**No. isolates**	**No. isolates with 100% reproducibility[a]** **(No. correctly identified)**	**No. isolates with 66% > 100% reproducibility[b]** **(No. correctly identified)**	**No. isolates with < 66 % reproducibility[b]**
ARA *E. coli* (N=9)	20	3 (1)	10 (2)	7
Ribotyping *E. coli* (N=3)	20	13 (2)	5 (2)	2
ARA *Enterococcus* (N=9)	19	3 (1)	8 (1)	8

[a]Number of isolates identified into the same category for all trials
[b]Number of isolates identified into the same category for at least 6 of 9 trials by ARA or 2 out of 3 trials by ribotyping

Table 11. Accuracy of ARA and Ribotyping for Classifying *E. coli* Isolates as Human and Sewage[a] vs. Non-human[b] (Animal-derived).

Predicted Source	ARA *E. coli*		Ribotyping *E. coli*		ARA *Enterococcus*	
	Source of Isolates					
	Human and Sewage (N=44)	Non-human (N=53)	Human and Sewage (N=44)	Non-human (N=53)	Human and Sewage (N=44)	Non-human (N=55)
Human and Sewage	14	12	22	10	29	25
Non-human	30	41	22	43	15	30
Sensitivity	32% (14/44)		50% (22/44)		66% (29/44)	
Specificity	77% (41/53)		81% (43/53)		55% (30/55)	
PPV[c]	54% (14/26)		69% (22/32)		54% (29/54)	
NPV[d]	58% (41/71)		66% (43/65)		67% (30/45)	
Accuracy	57% (55/97)		67% (65/97)		60% (59/99)	

[a]Combining human, sewage influent and effluent categories
[b]Combining cat, dog, seagull and horse categories
[c]Positive Predictive Value
[d]Negative Predictive Value

Table 12. Summary of average correct classification rates (ARCC) for ARA and ribotyping libraries and proficiency panels.

	ARCC (%) (no. isolates identified correctly/total no. isolates)	
	ARA	**Ribotyping**
E. coli Library	44% (1517/3477) [a]	64% (636/997) [a]
E. coli Proficiency Panel	29% (28/97) [a]	27% (26/97) [b]
Enterococcus Library	48% (1746/3657) [a]	Not done
Enterococcus Proficiency Panel	45% (45/99) [a]	Not done

[a] 6 category analysis: cat, dog, horse, seagull, human, sewage
[b] 7 category analysis: cat, dog, horse, seagull, human, influent, effluent

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